THIS NUMBER COMPLETES VOLUME VII

VOL. VII JUNE, 1919 No. 6

SOIL SCIENCE

FOUNDED BY

RUTGERS COLLEGE

NEW BRUNSWICK, N. J.

JAN 31920

JACOB G. LIPMAN, Editor-in-Chief

CARL R. WOODWARD, INGRID C. NELSON } Assistant Editors

IN CONSULTATION WITH

DR. F. J. ALWAY
University of Minnesota, St. Paul, Minn.
PROF. C. BARTHEL
Centralanstalten för Försökaväsendet på Jordbruksområdet, Stockholm, Sweden
DR. M. W. BEIJERINCK
Technische-Hoogeschool, Delft, Holland
PROF. A. W. BLAIR
Rutgers College, New Brunswick, N. J.
DR. P. E. BROWN
Iowa State College of Agriculture, Ames, Iowa
ALBERT BRUNO
Ministry of Agriculture, Paris, France
DIRECTOR H. R. CHRISTENSEN
Statens Planteavls-Laboratorium, Copenhagen, Denmark
DR. H. J. CONN
New York State Experiment Station, Geneva, N. Y.
DR. H. VON FEILITZEN
Svenska Mosskulturföreningen, Jönköping, Sweden
DR. E. B. FRED
University of Wisconsin, Madison, Wis.
DR. J. E. GREAVES
Utah Agricultural College, Logan, Utah
DR. R. GREIG-SMITH
Linnean Society, Sydney, New South Wales
DR. B. L. HARTWELL
Rhode Island Experimental Station, Kingston, R. I.
DR. C. B. LIPMAN
University of California, Berkeley, Calif.
DR. BURTON E. LIVINGSTON
Johns Hopkins University, Baltimore, Md.
DR. F. LÖHNIS
U. S. Department of Agriculture, Washington, D. C.
DR. T. L. LYON
Cornell University, Ithaca, N. Y.
DR. M. M. MCCOOL
Michigan Agricultural College, East Lansing, Mich.
DR. W. H. MCINTIRE
Tennessee Experiment Station, Knoxville, Tenn.
PROF. C. A. MOOERS
Tennessee Experiment Station, Knoxville, Tenn.
PROF. G. ROSSI
Royal Agricultural High School in Portici, Naples, Italy
DR. E. J. RUSSELL
Rothamsted Experiment Station, Harpenden, England
DR. O. SCHREINER
U. S. Department of Agriculture, Washington, D. C.
PROF. CHAS. E. THORNE
Ohio Experiment Station, Wooster, Ohio

PUBLISHED MONTHLY BY
WILLIAMS & WILKINS COMPANY
BALTIMORE, MD., U. S. A.

THE CAMBRIDGE UNIVERSITY PRESS
FETTER LANE, LONDON, E. C.

Entered as second-class matter May 12, 1919, at the post office at Baltimore, Maryland, under the act of March 3, 1879.

Copyright 1919, by Williams & Wilkins Company

Price { $6.00 per year, two volumes, United States, Mexico, Cuba
$6.25 per year, two volumes, Canada
$6.50 per year, two volumes, other countries

SOIL SCIENCE

Contents for June, 1919

E. B. Fred and E. J. Graul. Effect of Inoculation and Lime on the Amount of Nitrogen in Soybeans on Acid Soil 455

Emil Truog and M. R. Meacham. Soil Acidity: II. Its Relation to the Acidity of the Plant Juice 469

William J. Robbins and E. C. Lathrop. The Oxidation of Vanillin to Vanillic Acid by Certain Soil Bacteria 475

A. R. C. Haas. The Electrometric Titration of Plant Juices 487

Index 493

HENRY HEIL CHEMICAL CO.

210-214 South 4th Street

ST, LOUIS - MISSOURI

Importers and Manufacturers of

CHEMICAL APPARATUS, CHEMICALS

Assay and Laboratory Supplies Generally

We have the stock and can make prompt delivery

SPECIALTIES:

Kavalier Bohemian Normal Glassware; Best Grade of American Glassware, "Fry", "Non-Sol", "Perfection" and "Pyrex"; Max Dreverhoff, Schleicher & Schuell, Swedish and English Filtering Papers; Royal Berlin and Haldenwanger Berlin Porcelain Ware; Best American Porcelain Ware; Troemner & Becker Balances and Weights; Platinum Wire, Foil and Ware; Fused Silica Laboratory Utensils "Vitreosil"; Hanovia Pure Transparent Quartz Glass; Alundum Ware; Hoskins' Electric Furnaces; Le Chatelier's Pyrometers; Bausch & Lomb Microscopes and Accessories; Hydrometers and Thermometers of every description; Chemically Pure Acids and Ammonia, Molybdic Acid and Molybdate of Ammonia; Merck's, Baker & Adamson's and J. T. Baker Chemical Co.'s Chemicals and Reagents; Caustic Potash and Soda, purified in sticks and pure by alcohol; Acetate of Lead, Cyanide Mixture, Soda Ash and Commercial Caustic Soda.

EFFECT OF INOCULATION AND LIME ON THE YIELD AND ON THE AMOUNT OF NITROGEN IN SOYBEANS ON ACID SOIL[1]

E. B. FRED AND E. J. GRAUL

Departments of Agricultural Bacteriology and of Soils, University of Wisconsin

Received for publication June 30, 1919

In many parts of this state the soil is so low in calcium carbonate that the growth of alfalfa, or even clover, is almost a total failure (2). If the organic matter and nitrogen supply of these acid soils is to be maintained or increased, it is essential that leguminous crops be grown. Fortunately, there are certain legumes which are more tolerant of soil acidity than alfalfa and clover. Chief among these plants which may be of value in Wisconsin are soybeans, vetch, serradella and lupines.

As a soil builder the soybean has few equals. If grown in company with the nitrogen-fixing bacteria of the roots, this plant will absorb large amounts of nitrogen from the air. In other words, soybeans through the efforts of the bacteria, feed heavily upon the air nitrogen instead of upon the soil nitrogen (1). For the farmer on the light soils this property of the soybean is of great value. In the state of Wisconsin it is particularly important that soybeans be given a fair trial since it has been shown repeatedly that this plant will thrive in a soil which is moderately acid.

Several tests have been carried out with these crops on soils of varying reaction. The results of the work with soybeans are reported in this paper. In order to avoid confusion the technical information is given in simplified form.

The experiments reported in this paper were planned to study the influence that additions of limestone with and without inoculation with nodule bacteria exert upon the yield and composition of soybeans. In connection with this work the changes in the nitrogen supply of the soil were studied carefully.

Tests were carried out with soybeans on two important soil types—Plainfield sand and Colby silt loam. The results of both pot and field tests are included in this paper. In one pot test it was possible to make a complete nitrogen balance, i.e., to determine the amount of nitrogen in the soil at the beginning, and at the end, the amount in the crops, in the planted seed, and in the added water.

[1] Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

The sand soils were collected at Sparta and at Hancock, and the silt loam soils were collected at the Marshfield substation and at Curtiss. These soil samples were shipped to the laboratory where they were sieved and potted into 2- and 4-gallon glazed jars. In certain of the experiments, the results of the pot tests have been calculated in terms of pounds per acre. It is difficult to obtain a fair figure, and yet it has been shown repeatedly that pot tests, when properly controlled, give a true index of the nature of certain processes of the soil. For this calculation 1 gm. of plant tissue, or of nitrogen, to each jar of 10½ inches in diameter corresponds to 1 pound to each square rod or 160 pounds to each acre.

The fact that soybeans contain larger amounts of nitrogen than nearly any other plant, renders them especially valuable for forage. Moreover, it is well known that the greater part of this nitrogen is, or should be, obtained from the atmosphere. Thus, the ground on which soybeans are grown, instead of being impoverished, is actually enriched by the nitrogen residue. The experiments upon soybeans which have been carried on for many years at this and other agricultural experiment stations show that soybeans frequently grow well on soils which are moderately acid. In fact, they will often thrive on soils that are too acid to permit the growth of a profitable crop of red clover.

The authors have not found any evidence to support the statement that has been made repeatedly that tubercles in acid soils have little power to gather nitrogen from the air. Undoubtedly, an acid-reacting soil is injurious to the growth of many of the legume bacteria. However, when the bacteria have once gained entrance, and have formed nodules, the soil acidity has little if any effect on the extent to which nitrogen is drawn from the air.

SOYBEANS ON SPARTA SAND

In the spring of 1915 twelve large jars, each containing 19,150 gm. of Sparta sand, were planted to mammoth red clover. Aside from the lime treatments, all of the jars were given a liberal amount of phosphorous and potassium in order that the lack of these elements would not limit crop growth. From these jars three cuttings were made. The benefits from lime and from nitrogen-gathering bacteria on the yield and amount of nitrogen in clover cannot be questioned. Lime at the rate of 3.2 tons per acre, enough to neutralize only half of the soil acidity, increased the growth of red clover more than 50 per cent, as is shown in table 1.

A summary of the results of the clover crop are shown in table 1.

Twenty-six hundredths of a gram of lime to 100 gm. of soil is equal to 3.2 tons per acre, or one-half enough to neutralize all of the active soil acids. Correspondingly, 0.52 gm. is equal to 6.5 tons, or enough to neutralize all of the active soil acids.

TABLE 1

Effect of inoculation and of lime on clover

NUMBER	TREATMENT	LIME ADDED TO 100 GM. OF SOIL	TOTAL DRY WEIGHT OF TOPS	TOTAL NITROGEN IN TOPS	
		gm.	*gm.*	*mgm.*	*per cent*
1	Uninoculated	0	48.55	1604.5	3.31
2	Inoculated	0	53.86	1945.6	3.61
3	Uninoculated	0.26	73.26	2473.8	3.38
4	Inoculated	0.26	76.78	2614.6	3.41
5	Uninoculated	0.52	70.63	2276.9	3.22
6	Inoculated	0.52	77.08	2510.7	3.26

Although red clover has been grown for many years on this soil type it appears that the soil is not thoroughly infected with the nodule organisms. In every case the jars to which the bacteria were added showed a gain in dry matter and in total nitrogen. Possibly the legume bacteria present in these soils are not so active as the bacteria added in the inoculum.

TABLE 2

Effect of inoculation and of lime on soybeans

NUMBER	TREATMENT	LIME ADDED TO 100 GM. OF SOIL	TOTAL DRY WEIGHT OF TOPS*	TOTAL NITROGEN IN TOPS	
		gm.	*gm.*	*mgm.*	*per cent*
1	Uninoculated	0	31.4	1006.0	3.21
2	Inoculated	0	38.9	1469.0	3.78
3	Uninoculated	0.26	52.5	1517.9	2.89
4	Inoculated	0.26	54.7	1898.2	3.47
5	Uninoculated	0.52	44.0	1402.3	3.19
6	Inoculated	0.52	52.6	1920.9	3.65

* Two crops.

In the spring of 1916, without further treatment this Sparta sand was planted to soybeans. The young seedlings were inoculated with a pure culture of bacteria isolated from a soybean nodule. The soybeans made only a fair growth. The plants were cut, and the roots examined for nodules. The inoculated roots showed an abundance of nodules, except in jar 2, the unlimed sand. Here only a few nodules were found. Since the culture used for the inoculation undoubtedly contained millions of active nodule bacteria, it is strange that nodules were not found on the roots of these plants. Perhaps the acid reaction of the soil so injured the bacteria that they were unable to enter the host plant.

The roots of the first soybean crop were returned to the soil and the sand replanted to soybeans. Inoculation was repeated. The second crop made a luxuriant growth. The plants were more than 3 feet tall when harvested.

In every case the inoculated plants were of a deeper green color than the uninoculated plants. The roots were examined for nodules and returned to the soil. All of the plants in the uninoculated soil were free of nodules while the roots in the inoculated soil were thickly studded with nodules. A summary of the results of this study is given in table 2. Unless stated otherwise, the figures represent the average of two or three jars. In many cases two or three crops were grown in each jar.

The inoculated plants showed a gain in dry matter of 7.6 gm., or 24.2 per cent increase as compared with the uninoculated plants. Where lime and inoculation were used together the increase was 23.4 gm. of dry matter, or 74.7 per cent. It is of particular interest to note the effect of varying amounts of lime. Lime sufficient to neutralize one-half of the soil acidity gave the best results.

Besides this gain in dry matter, the inoculated plants contained a higher percentage of nitrogen. Here, as in many other tests, the benefit from the inoculation of soybeans is more noticeable in the gain in nitrogen than in increased crop yield.

SOYBEANS ON HANCOCK SAND

This soil was secured from the Hancock substation. The field from which the soil was secured had been cropped for a number of years without the addition of any fertilizer. Consequently, the soil was in a poor state of fertility. It is not known definitely, but it is improbable that soybeans had ever been grown on this soil. The results of the experiments support this statement. The soil is a light brown sand with little organic matter, and is decidedly acid in reaction. Since this sand is very low in phosphorus and potassium, an application of 0.25 gm. of dibasic potassium phosphate (K_2HPO_4) per kilogram of dry soil was made.

According to the results of acidity tests, this sand would require 2.575 tons of calcium carbonate to neutralize the acid in one acre of 2,500,000 pounds. In the experiments that follow it was arranged to study the effect of different amounts of lime on soybeans. Three amounts of calcium carbonate were used: (a) 0.105 gm. to each 100 gm. of sand (sufficient to neutralize one-half of the active soil acidity), (b) 0.210 gm. to each 100 gm. (sufficient to neutralize all of the active acidity), and (c) 0.315 gm. to each 100 gm. (one and one-half times the amount required to neutralize the active acidity).

The sand was put into 2-gallon jars, 10,940 gm. to each jar, and water added to bring the soil to 60 per cent of saturation. As far as possible the soil moisture was maintained at this point.

On June 24 the jars were planted to Wisconsin Black soybeans. Before planting, the seeds were treated with warm mercuric chloride solution to remove any nodule bacteria. As soon as the young seedlings appeared, the soil was inoculated with a pure culture of the soybean bacteria. The roots

of the plants were examined from time to time by carefully washing away a small amount of sand from one of the plants. Although this is by no means an accurate test, it furnishes information concerning the approximate date of inoculation. Eighteen days afterward, the inoculated plants showed profuse nodule formation, but no apparent difference from the uninoculated in growth. However, one week later the inoculated plants were somewhat deeper green in color than the uninoculated, and slightly larger. The soybeans were cut August 20. The tops were saved for analysis, and the roots

TABLE 3

Effect of inoculation and of lime on soybeans

NUMBER	TREATMENT	LIME ADDED TO 100 GM. OF SOIL	TOTAL DRY WEIGHT OF TOPS	TOTAL NITROGEN IN TOPS	
		gm.	*gm.*	*mgm.*	*per cent*
1	Uninoculated	0	8.9	135.7	1.53
2	Inoculated	0	20.0	639.3	3.20
3	Uninoculated	0.105	14.0	235.4	1.68
4	Inoculated	0.105	21.7	721.5	3.32
5	Uninoculated	0.210	13.3	243.1	1.83
6	Inoculated	0.210	18.1	599.3	3.31
7	Uninoculated	0.315	13.9	256.8	1.85
8	Inoculated	0.315	14.2	448.7	3.16

TABLE 4

Effect of inoculation and of lime on yield and on composition of soybeans

NUMBER	TREATMENT	LIME ADDED PER ACRE	DRY WEIGHT PER ACRE		NITROGEN PER ACRE	
			Total	Increase due to treatment	Total	Increase due to treatment
		lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*
1	Uninoculated	0	2296.2		35.0	
2	Inoculated	0	5160.0	2863.8	164.9	129.9
3	Uninoculated	2625	3612.0	1315.8	60.7	25.7
4	Inoculated	2625	5598.6	3302.4	186.1	151.1
5	Uninoculated	5250	3431.4	1135.2	62.7	27.7
6	Inoculated	5250	4669.8	2373.6	154.6	119.6
7	Uninoculated	7875	3586.2	1290.0	66.3	31.2
8	Inoculated	7875	3663.6	1367.4	115.8	80.8

were examined. The roots from the uninoculated soil were entirely free from nodules while from inoculated soils the roots were thickly studded with large and small nodules. All of the root tissue was returned to the soil.

On March 1 the following spring, the jars were replanted to soybeans. This second crop made only a fair growth. As in the case of the preceding crop, the plant stems and leaves were removed for analysis, and the roots returned to the soil. Before planting the third crop 0.2 gm. of dibasic sodium phosphate (Na_2HPO_4) to each kilogram of soil was added to each jar. On June

2 soybeans were sown and the soil re-inoculated with soybean bacteria. The plants grew better than any of the preceding crops. Three weeks after seeding, the inoculated plants were clearly marked by a deep green color. The uninoculated control remained yellow, while the plants from the limed jars exhibited a green color somewhat lighter in shade than the plants from the inoculated jars. This third and best crop of soybeans was cut July 25, and the tops saved for analysis. On examination of the roots it was found that the uninoculated control jars were free from nodules. The uninoculated and limed soils showed very few if any nodules; whereas the inoculated soils showed an abundance of nodules. The results of this study involved a great number of analyses and considerable data. Obviously, it is preferable to condense the results. Therefore, only the average values are here presented.

Tables 3 and 4 give the results of the first crop yield and nitrogen content of soybeans grown under the four conditions: no inoculation and no lime, inoculation only, lime only, and inoculation plus lime. The lime was applied in three amounts. The figures of table 4 are the same as those of table 3 except that they are calculated to the acre basis. It will be seen from the table that inoculation was far more effective than lime in promoting the growth of soybeans on Hancock sand; although lime alone caused an increase in the crop. It is true that lime and inoculation together gave the maximum yield; however, the difference is not great. The gain in the crop from lime alone is most pronounced in the case of the smallest application. Larger amounts of lime were favorable, but not in proportion.

The determination of the total as well as of the percentage of nitrogen brings out some interesting points. Perhaps the most noteworthy fact is the enormous accumulation of nitrogen in the inoculated plants as compared with that in the uninoculated plants. Here, as in many other experiments, the influence of inoculation on soybeans is noted chiefly in the greater nitrogen accumulation, rather than in any marked gain in the yield of dry matter. The favorable influence of lime on total nitrogen may be accounted for by the increase in soluble nitrogenous compounds of the soil—a process which is well known.

Turning now to the effect of inoculation on the percentage of nitrogen in the crop, the results are most striking. Inoculation alone more than doubled the percentage of nitrogen in soybeans. The application of lime had little effect. Table 5 gives the results of all three crops. The whole evidence points to the importance of having the proper bacteria growing with soybeans if the maximum amount of nitrogen and the largest yields are to be secured. In the absence of bacteria soybeans take large amounts of nitrogen from the soil.

A study has been made of the source and the amount of nitrogen in soybeans with and without nodule bacteria when grown under varying conditions of reaction. For this purpose a careful record has been kept of the amount of nitrogen added to the soil in the seed, and of the nitrogen removed in each

crop. Determinations of the total nitrogen in the soil at the beginning and at the end have been made. The results of these analyses are shown in table 6. These figures represent three successive crops of soybeans on Hancock sand. The results show clearly that nodule bacteria increase the total amount and the percentage of nitrogen in soybeans. In order to emphasize the nitrogen nutrition of soybeans when grown alone and in company with bacteria, the figures of table 6 were used in drawing figure 1. Here the source of nitrogen for soybeans can be seen at a glance.

TABLE 5

Effect of inoculation and of lime on soybeans

NUMBER	TREATMENT	LIME ADDED TO 100 GM. OF SOIL	TOTAL DRY WEIGHT OF TOPS*	TOTAL NITROGEN IN TOPS*	
		gm.	*gm.*	*mgm.*	*per cent*
1	Uninoculated	0	28.5	540.1	1.89
2	Inoculated	0	51.2	1759.6	3.44
3	Uninoculated	0.105	47.5	1292.0	2.70
4	Inoculated	0.105	57.7	1982.2	3.44
5	Uninoculated	0.210	41.8	1099.3	2.63
6	Inoculated	0.210	53.2	1749.6	3.29
7	Uninoculated	0.315	47.7	1213.2	2.54
8	Inoculated	0.315	47.3	1507.8	3.19

* Three crops.

TABLE 6

The source of nitrogen in soybeans

NUMBER		AVERAGE TOTAL NITROGEN PER ACRE			
		Uninoculated	Inoculated	Uninoculated plus lime	Inoculated plus lime
		lbs.	*lbs.*	*lbs.*	*lbs.*
1	Soil at beginning	1165.0	1165.0	1165.0	1165.0
2	Soil at end	1016.5	1034.6	1003.6	1042.3
3	Taken from soil	148.5	130.4	161.4	122.7
4	Crops, leaves and stems	139.3	453.9	333.2	511.7
5	Taken from air	9.2	323.5	171.8	389.0

From this diagram it will be seen that if the portion above ground of a well inoculated legume is removed there is an actual loss in total nitrogen. Although a large part of the nitrogen may be taken from the air, it is wrong to suppose that the crop, i.e., the tops, may be cut, removed, and yet the soil supply of nitrogen be maintained. The evidence obtained from many experiments does not in any way indicate that the roots of legumes excrete appreciable quantities of nitrogenous compounds. In farm practice the result would be different; since the farmer would hardly remove all of the plant tissue, a certain portion would at least be returned to the land either as plant residue or as manure.

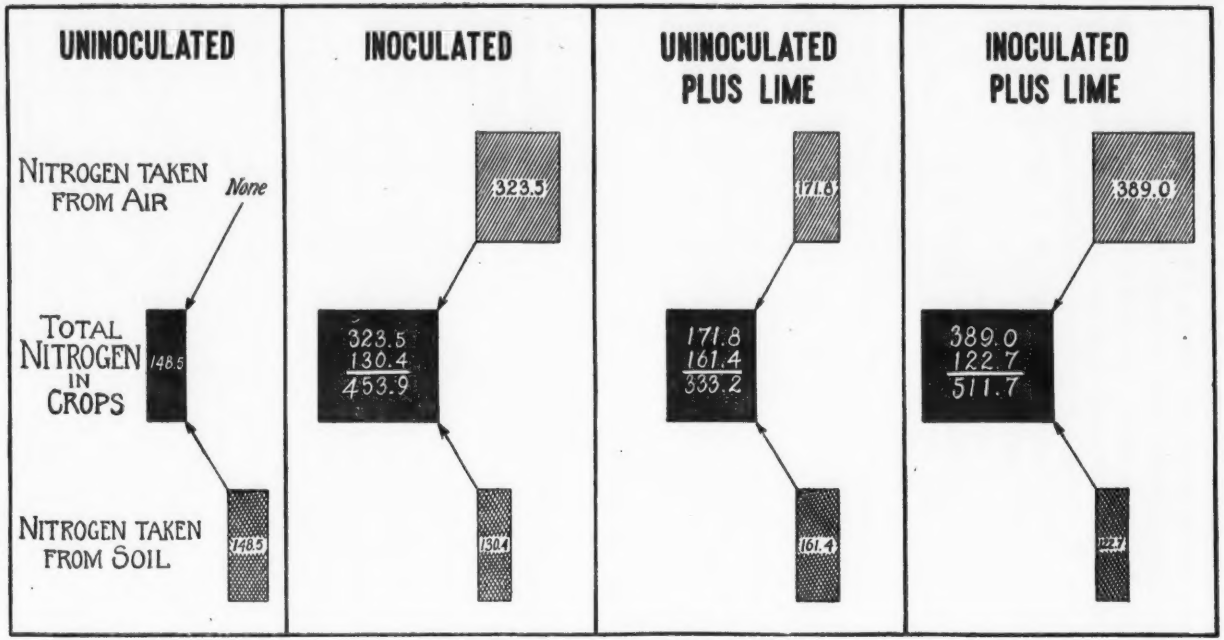

FIG. 1. SOURCE OF NITROGEN FOR SOYBEANS

SOYBEANS ON COLBY SILT LOAM FROM MARSHFIELD

In these tests the same methods were followed as previously outlined. In the spring of 1915 the jars of Colby silt loam soil were planted to red clover. This clover was cut three times and at each cutting the tops were removed for analysis, whereas the roots were allowed to remain in the soil. The yield of the clover in dry matter, and the amount of nitrogen as well as the percentage of nitrogen, are shown in table 7. In general, inoculation failed to

TABLE 7

Effect of inoculation and of lime on red clover

NUMBER	TREATMENT	LIME ADDED TO 100 GM. OF SOIL	TOTAL DRY WEIGHT OF TOPS	TOTAL NITROGEN IN TOPS	
		gm.	*gm.*	*mgm.*	*per cent*
1	Uninoculated	0	56.56	1998.0	3.54
2	Inoculated	0	55.53	2034.7	3.66
3	Uninoculated	0.51	62.90	2279.3	3.62
4	Inoculated	0.51	59.54	2246.7	3.78
5	Uninoculated	1.02	61.58	2317.1	3.76
6	Inoculated	1.02	64.97	2386.4	3.68

TABLE 8

Effect of inoculation and of lime on soybeans

NUMBER	TREATMENT	LIME ADDED TO 100 GM. OF SOIL	TOTAL DRY WEIGHT OF TOPS	TOTAL NITROGEN IN TOPS	
		gm.	*gm.*	*mgm.*	*per cent*
1	Uninoculated	0	65.0	1822.6	2.81
2	Inoculated	0	61.0	2043.9	3.35
3	Uninoculated	0.51	57.3	1597.2	2.79
4	Inoculated	0.51	64.5	2238.2	3.47
5	Uninoculated	1.02	62.0	2007.0	3.24
6	Inoculated	1.02	64.6	2249.9	3.48

increase the crop yield. Apparently Colby soil at Marshfield is well supplied with active clover bacteria. Lime alone caused a slight stimulation in growth of clover. The results show that this soil type may be decidedly acid and yet produce a good crop of red clover.

The following year, March 13, 1916, these jars were planted to Wisconsin Black soybeans. Four days later the inoculated jars of soil were treated with a water suspension of soybean bacteria. The plants made a good growth, but without any apparent difference between the uninoculated and the inoculated jars. On May 10 this first crop of soybeans was cut. The tops were saved for analysis, whereas the roots were examined for nodules and returned to the soil. The roots of the uninoculated plants were free of nodules while the inoculated were thoroughly infected. A second crop was planted May 20,

and the inoculated jars of soil again seeded with soybean bacteria. The plants grew rapidly and a large yield was obtained. Unfortunately, the uninoculated jars of soil became slightly inoculated.

A summary of the results of the nitrogen determinations as well as dry weight of the two crops is shown in table 8. The data of this table deserve careful consideration. Inoculation did not cause any decided change in the yield of soybeans, but it did bring about a marked increase in the nitrogen content of the plant tissue. In every case, the nitrogen-gathering bacteria brought about a gain in the total amount and in the percentage of nitrogen in the crops. Lime and inoculation favored the growth of the soybeans, and to a slight degree, the fixation of nitrogen.

SOYBEANS ON COLBY SILT LOAM FROM CURTISS

For more than forty years this soil at Curtiss had been cropped and no commercial fertilizer or lime returned. To 13,688 gm. per jar of this cropped soil, sodium phosphate and calcium carbonate were added as indicated in table 9. One hundred grams of this soil required 0.848 gm. of limestone to

TABLE 9

Effect of inoculation, of lime, and of phosphorus on soybeans

NUMBER	TREATMENT	ADDED TO 100 GM. OF SOIL		TOTAL DRY WEIGHT OF TOPS	TOTAL NITROGEN IN TOPS	
		Lime	Na_2HPO_4			
		gm.	*gm.*	*gm.*	*mgm.*	*per cent*
1	Uninoculated	0	0	33.28	592.8	1.78
2	Inoculated	0	0	32.01	780.3	2.44
3	Inoculated	0	0.0219	36.86	1063.8	2.89
4	Uninoculated	0.212	0.0219	37.76	674.0	1.78
5	Inoculated	0.212	0.0219	38.19	1138.0	2.98
6	Uninoculated	0.424	0.0219	40.88	805.4	1.97
7	Inoculated	0.424	0.0219	39.58	1284.7	3.25
8	Uninoculated	0.848	0.0219	39.27	1223.2	3.11
9	Inoculated	0.848	0.0219	42.38	1473.4	3.47

neutralize its acidity. The data of this experiment are given in the figures of summary table 9. Examination of the plant roots from uninoculated jars of soil showed the absence of nodules, while roots in inoculated jars of soil were well covered with nodules. It will be seen from the figures of the table that inoculation alone did not increase the crop yield. Apparently, small amounts of lime and phosphorus coupled with inoculation favored plant growth, and gave the most profitable yield.

Turning to the results of the nitrogen analyses, the effect of inoculation is brought out in a very striking way. In every case inoculation increased the amounts of nitrogen in the plant tissue. It is also true that the addition of lime was favorable to an increased nitrogen content of the soybean seed.

SOYBEANS AT THE MARSHFIELD SUBSTATION

A series of plats were planted to Wisconsin Black soybeans as shown in the plan of table 10. No attempt was made to add the limestone on the basis of acid present in the soil. Instead the lime was applied in varying amounts from 1 to 8 tons per acre. Approximately 8.5 tons of lime were required to neutralize all of the soil acids present. The plats were $\frac{1}{60}$ of an acre in size and were arranged so as to have a control plat near one end of the group, and another near the middle of the group. Duplicate plats were used for each test.

When the plants were a few weeks old, tubercles were found in great numbers on the plants from the inoculated soils, while plants from the uninoculated soils showed only an occasional nodule. One of the control plats became

TABLE 10

*Effect of inoculation and of lime on soybeans**

NUMBER	TREATMENT	LIME ADDED PER ACRE	DRY WEIGHT OF TOPS		DRY WEIGHT OF SEEDS	TOTAL NITROGEN OF TOPS		TOTAL NITROGEN OF SEEDS	NITROGEN OF TOPS: SEEDS REMOVED	NITROGEN OF SEEDS
			With seeds	Without seeds		With seeds	Without seeds			
		lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*per cent*	*per cent*†
1	Uninoculated...		3172.0	2795.8	376.2	75.2	45.8	28.4	1.64	7.54
2	Inoculated.....		3294.4	2899.8	394.6	80.5	51.6	28.9	1.78	7.33
3	Uninoculated...	2,000	2756.2	2352.1	404.1	62.3	33.1	29.2	1.42	7.21
4	Inoculated.....	2,000	3450.6	2986.2	464.4	89.1	54.5	34.6	1.86	7.49
5	Uninoculated...	6,000	2693.2	2411.1	282.1	53.2	32.5	20.7	1.36	7.45
6	Inoculated.....	6,000	3702.6	3303.9	398.7	91.2	61.5	29.7	1.86	7.44
7	Uninoculated...	16,000	3424.0	2990.2	433.8	75.8	43.0	32.8	1.45	7.52
8	Inoculated.....	16,000	3955.4	3460.9	494.5	99.9	63.3	36.5	1.83	7.39

* Acre basis.

† Average percentage.

infected to a considerable degree, but it is not surprising that the uninoculated plats became slightly infected when we consider that there were heavy rains, and that the plats were level. The influence of the soil treatment is well illustrated from the results presented in table 10. Inoculation alone, and inoculation with the addition of lime stimulated the growth of soybeans. Far more important than the difference in yield is the increase in nitrogen of the inoculated plants. Here, as in previous experiments, the tops of the plants inoculated with nitrogen-gathering bacteria gave a higher percentage of nitrogen than the tops of the uninoculated plants. The nitrogen-fixing bacteria also increased the dry weight of the seeds, and the total nitrogen content of the seed, but apparently did not increase the percentage of nitrogen in the seeds.

Concerning the effect of lime, it appears that between 1 and 3 tons to each acre gives the most profitable returns. Larger quantities, such as 8 tons to

the acre, failed to cause any marked change in the yield. From these and from other similar experiments it is concluded that soybeans should be inoculated when first seeded, and as a rule, they should be grown a second time on the same land.

SOYBEANS AT CURTISS

In the spring of 1917, twenty plots were arranged on cropped Colby silt loam and on May 10 planted to Wisconsin Black soybeans. The plots were $\frac{1}{150}$ acre in size. The summer was very dry, and an early frost damaged the crop severely. It was harvested September 1, and samples were drawn for analysis. The treatments of the different plots, together with the crop yields and nitrogen content, are recorded in table 11. Here as in many other field and pot experiments there is but little increase in the crop production on the addition of nitrogen-fixing bacteria to such soils.

TABLE 11

*Effect of inoculation and of lime on soybeans**

NUMBER	TREATMENT	BONE MEAL ADDED PER ACRE	LIME ADDED PER ACRE	TOTAL DRY WEIGHT OF TOPS	TOTAL NITROGEN IN TOPS	
		lbs.	*lbs.*	*lbs.*	*lbs.*	*per cent*
1	Uninoculated	400		2622.9	83.2	3.17
2	Inoculated	400		2425.1	84.7	3.51
3	Uninoculated	400		2496.0	83.0	3.33
4	Inoculated	400		2452.2	88.0	3.60
5	Uninoculated	400	2,000	2417.5	81.0	3.36
6	Inoculated	400	2,000	2749.7	95.8	3.53
7	Uninoculated	400	6,000	2796.5	96.3	3.43
8	Inoculated	400	6,000	2875.0	110.9	3.87
9	Uninoculated	400	10,000	2736.1	94.7	3.46
10	Inoculated	400	10,000	2943.0	107.8	3.69

* Acre basis.

SUMMARY

It is noteworthy that in every test the percentage of protein in the tops was higher in the case of the nodule-bearing plants than with the plants free of nodules. Although the absence of the nodule bacteria renders it impossible for the soybean plant to utilize the nitrogen of the air, the effect is not always apparent. In parts of Wisconsin, soybeans will produce large crops in the absence of the nodule bacteria, but in such cases, the entire nitrogen supply of the plant is taken from the soil.

Inoculation of soybeans grown on Sparta sand increased the yield of dry matter. The yield was further increased when inoculation was supplemented by an application of lime. Sufficient lime to neutralize one-half of the active soil acidity was sufficient to produce maximum yields. The percentage of nitrogen in the crop and the total amount was increased by inoculation.

The crops grown on the sand from Hancock yielded better when inoculated than when uninoculated. In fact, inoculation was more effective in increasing the yield than was lime. Lime in small amounts and inoculation together produced the best yields.

The nitrogen content of those crops which were inoculated was considerably higher than that of the uninoculated crops. When the crops are not inoculated all of the nitrogen is drawn from the soil, but when the proper bacteria are present a large proportion of the protein nitrogen is drawn from the air.

When the entire crop is removed from the soil, leaving only the roots and stubble, there will be less nitrogen in the soil than there was before the crop was grown, but if the crop is turned under as a green manure, if properly inoculated, it will add large quantities of nitrogen to the soil.

Inoculation was not as effective on the Colby silt loam as on the sandy soils in increasing yields, but influenced the percentage of nitrogen, and consequently the total amount in the crop.

Soybeans if properly inoculated will utilize nitrogen from the air and thus enrich the soil, provided the crop residue is returned to the soil. It is not necessary to plow the crop under if the crop is fed on the farm. The manure can be returned to the soil, and in this way be utilized in the improvement of the land. Small additions of limestone to acid soils render the inoculated soybean plant more active in absorbing air nitrogen.

REFERENCES

(1) FELLERS, C. R. 1918 The effect of inoculation, fertilizer treatment, and certain minerals on the yield, composition, and nodule formation of soybeans. *In* Soil Sci., v. 6, no. 2, p. 81–119.

(2) FRED, E. B., AND GRAUL, E. J. 1916 The gain in nitrogen from growth of legumes on acid soil. Wis. Agr. Exp. Res. Bul. 39.

SOIL ACIDITY: II. ITS RELATION TO THE ACIDITY OF THE PLANT JUICE[1]

EMIL TRUOG AND M. R. MEACHAM

Department of Soils, Agricultural Experiment Station, University of Wisconsin

Received for publication July 3, 1919

In the first paper (7) of this series the general relation of soil acidity to plant growth was discussed. It was stated, with certain reservations, that the main specific harmful influence of soil acidity on certain plants is that it prevents these plants from securing at a sufficiently rapid rate the calcium as the carbonate or bi-carbonate which is needed to neutralize and precipitate certain acids in the plants themselves, which are probably largely by-products produced as the result of certain vital reactions in the growth of plants. If calcium in these forms is not furnished at a sufficiently rapid rate, then the rate of these reactions is lowered accordingly, as is also the rate of plant growth. If this is the case, then it seems that the juice of at least some plants growing on acid soils should be slightly more acid than that of plants growing on non-acid, or limed acid soils. That this condition usually holds was briefly stated in the first paper. The data previously referred to relating to this phase of the subject are given in this paper.

Except where otherwise stated, the plants were grown in 2-gallon pots in the greenhouse on limed and unlimed medium, to strongly acid, soils. In the limed series, crushed limestone was added, according to the acidity, in amounts equivalent to common field applications. The determinations on the corn plants were made when the plants were about 15 inches high. The other plants were used shortly before blooming.

With three exceptions, later given, the juice was secured as follows: The plants were cut within several inches of the ground and thoroughly macerated by passing them through a tinned meat grinder. The juice was then expressed with a press. In most cases before making the determination the juice was centrifuged about 5 minutes to remove coarse material in suspension. This was found more satisfactory than filtration. The determinations were then made immediately on this juice without the addition of water.

In three cases of alfalfa—(a), (b) and (c), table 1, the juice was obtained in a different manner from that just explained. In the cases of (a) and (b) 10 gm. of the plant tops were thoroughly macerated and then worked up with 100 cc. of water and filtered. The filtered extract was then used. In

[1] Published with the permission of the director of the Wisconsin Agricultural Experiment Station.

the case of (c), 15 gm. of the tops were macerated with 10 gm. of quartz, and the juice pressed out. Of this juice 5 cc. were diluted with 50 cc. of water, and the determination made on this. These three determinations were made in 1916 with the assistance of N. E. Loomis.

The determinations of the acidity,[2] or hydrogen-ion concentrations, were made electrometrically at 25°C. with an apparatus slightly modified from that described by Loomis (4) and Acree. The results, or acidity, are expressed in terms of the P_H symbol of Sorensen. Corrections were made for pressure and variations of the calomel electrode as suggested by Loomis (5) and Acree.

TABLE 1

The acidity in terms of hydrogen-ion concentration of the juice of plants (tops) grown on unlimed and limed acid soils

KIND OF PLANT	P_H VALUE OF THE JUICE	
	Unlimed acid soil	Limed acid soil
Alfalfa, *Medicago sativa* (a)	5.78	6.03
Alfalfa, *Medicago sativa* (b)	5.79	5.88
Alfalfa, *Medicago sativa* (c)	6.28	6.46
Alfalfa, *Medicago sativa* (d)	5.61	5.67
Alfalfa, *Medicago sativa* (e)	5.80	5.79
Alfalfa, *Medicago sativa* (f)	5.62	5.57
Clover, *Trifolium pratense* (a)	5.69	5.83
Clover, *Trifolium pratense* (b)	5.81	5.90
Clover, *Trifolium pratense* (c)	5.69	5.74
Clover, *Trifolium pratense* (d)	5.73	5.80
Lupine blue, *Lupinus hirsitus* (a)	5.54	5.45
Lupine blue, *Lupinus hirsitus* (b)	5.65	5.45
Soybean, *Glycine hispida*	5.81	5.94
Buckwheat, *Fagopyrum esculentum*	4.02	4.05
Corn, *Zea mays*	5.21	5.31
Rape, *Brassica napus*	5.10	5.18

In table 1 the results of the limed and unlimed series are given. In 12 cases out of 16 the plant juices are more acid on the unlimed series. Only in 2 cases out of 6 with alfalfa and in the case of lupine are the results reversed. The blue lupine is sometimes injured by liming; and hence an important question is raised whether or not certain injuries result in an increase in acidity. In the two reversed cases of alfalfa (e), and (f), the differences are quite small and it is possible that the reversed results may be due to small differences which existed in moisture supply, time of day plants were cut, and way in which juice was handled, although some attempt was made to make these conditions uniform. It is to be noted that the acidity of both the limed and unlimed alfalfa in these cases is quite high for alfalfa, and that evidently even in the limed series the plants were not getting sufficient lime.

[2] In this paper acidity and hydrogen-ion concentration are used synonymously.

The results given in table 2 emphasize this point. The alfalfa from which the results of table 2 were obtained was grown out-of-doors on a rich neutral soil, and the acidity of the plant juice is much less than in the two reversed cases.

The data in table 2 were obtained in studying the effect of various conditions of determination indicated in the table, on the acidity of the plant juice. Number 2 is considerably more acid than number 1, indicating that the expressed juice becomes more acid on standing. The values obtained in numbers 1 and 3 while the weather was warm and dry indicate a slightly higher acidity than the comparable values in numbers 4 and 7 obtained after a heavy rain.

TABLE 2

Effect of various conditions of determination on the acidity in terms of hydrogen-ion concentration of alfalfa (tops) juice

Number	CONDITIONS OF DETERMINATION					P_H VALUES
	Date	Weather	Time of day when cut	Time when juice was expressed	Time when determination was made	
1	May 18	Clear, warm, dry	6.00 a.m.	8.00 a.m.	8.30 a.m.	5.97
2	May 18	Clear, warm, dry	6.00 a.m.	8.00 a.m.	5.12 p.m.	5.86
3	May 18	Clear, warm, dry	2.00 p.m.	2.30 p.m.	3.45 p.m.	6.00
4	May 19	Heavy rain previous night, warm	8.00 a.m.	8.30 a.m.	9.45 a.m.	6.04
5	May 19	Heavy rain previous night, warm	8.00 a.m	10.30 a.m.	10.59 a.m.	6.05
6	May 19	Heavy rain previous night, warm	8.00 a.m.	1.00 p.m.	1.40 p.m.	6.18
7	May 19	Heavy rain previous night, warm	2.30 p.m.	2.45 p.m.	2.57 p.m.	6.11
8	May 19	Heavy rain previous night, warm	2.30 p.m.	3.30 p.m.	4.00 p.m.	6.10

A comparison of number 1 with number 3, and number 4 with number 7, shows that juice from plants cut in the morning is more acid than that from plants cut later in the day, indicating an accumulation of acids at night which were partly used up in the day time, due to diurnal changes in plant processes. A comparison of numbers 4, 5, and 6 indicates that when plants are cut and kept several hours before expressing the juice and making the determination, the acidity may become slightly less.

These data in table 2 emphasize the importance of standardizing the conditions of making the determinations, especially when small differences are to be measured and used in a comparative way. In studying the effect of one factor, like liming, on the acidity of plant juice, other factors like moisture supply, time of day material is cut, and methods of handling the juice, must be made uniform. It appears that after a plant is cut, and especially

after the juice is expressed, the determination should be made as quickly as possible.

Another factor in this connection is the question whether or not the roots and tops of the same plant are of the same acidity Three comparative sets of determinations were made, and are given in table 3. In two cases the roots are slightly more acid than the tops and in the third case the tops are more acid. In this latter case, which is with alfalfa, the plants were grown in pots on a strongly acid soil, and the acidity of both the tops and roots is high for alfalfa, since an acidity of about P_H. 6.0 is normal for alfalfa, as indicated by the other set of figures, and also those in table 2 in which cases the alfalfa was grown out-of-doors on a neutral soil.

TABLE 3

Acidity in terms of hydrogen-ion concentration of the juice of tops and roots

KIND OF PLANT	KIND OF SOIL	P_H VALUES OF THE JUICE	
		Tops	Roots
Clover	Acid	5.74	5.71
Alfalfa	Neutral	6.00	5.84
Alfalfa	Acid	5.61	5.72

The clover was also grown in pots on an acid soil but the acidity found is not far from normal for clover. Apparently the roots are often more acid than the tops, although the reverse may also be the case. More determinations are needed to show how the different parts of the same plant vary in acidity. The results of Kappen (3) show a higher acidity in the tops than in the roots, both as regards actual and total acidity.

The importance of a proper regulation of the acidity of plant juices or protoplasm has been indicated by Kappen (3) who states that the plant juice acidity of each species of plant is specifically adapted to the physiological processes of that plant. The effect of acidity on plant enzyme action is emphasized by him.

The importance of a proper actual plant acidity on oxidase activity has been pointed out by Reed (6) and Bunzell (1). Some unusually high acidities of plant tissues or protoplasm have been reported by Haas (2), who worked largely with fruits. These results indicate that some life processes go on normally in the presence of a high acidity.

Kappen made acidity determinations by means of the gas chain, and also by titration of a considerable number of plant roots and some tops. It is in the green tops and in the leaves especially that the most active life processes take place; and hence the determinations reported in this paper were made almost entirely on the tops. Kappen made one set of comparative determinations on limed and unlimed plant roots, and that was with the yellow lupine. This was made to study the lime sensitiveness of the lupine.

He found the limed plant roots less acid than the unlimed. This is opposite to what the writers found in the tops of the blue lupine.

A relation between plant juice acidity and disease conditions and immunity has been indicated by Wagner (8). Soil acidity, by affecting plant juice acidity, may have a relation to certain plant diseases. This phase of the subject deserves further investigation.

SUMMARY

There are considerable differences in the acidities of the juices of different species of plants. The acidity of each species of plant, while it may vary to an extent easily measurable, is however, usually limited to a rather narrow range. Undoubtedly for each species of plant there is a certain acidity which is most favorable for the life processes of that species. Unquestionably in many cases soil acidity, by limiting the supply of lime available for plants, affects the acidity of the juice or protoplasm of these plants. The importance of a proper regulation of plant acidity in relation to vital plant processes should be noted.

In the life processes of plants, acids are formed, some of which are probably simple by-products. Lime and other bases are needed to neutralize these acids. Plants high in protein are usually high in lime and other bases indicating the formation of acids in protein synthesis. If the supply of lime and other bases is inadequate, the acidity of the plant juice rises to a certain extent which is limited because the accumulation of acids probably decreases the rate of the processes which produce acids. A condition of self regulation thus probably exists and death due to over-acidity is prevented. Slow growth and a weakened condition, however, result as is the case with plants of high lime requirement growing on acid soils.

In plants there are many "buffer" substances which to a certain extent help to maintain a more uniform acidity and prevent rapid and exeessive alterations, as would result especially from diurnal changes in plant processes. Bases are however usually needed in the formation of these "buffer" substances.

There are many conditions which affect the acidity of the plant juice. Some of these conditions are indicated in this paper. Before final conclusions can be made regarding this subject, much more investigation is needed. The proper method of carrying on this investigation, and especially the technique of making the acidity determinations, needs to be standardized. An effort to do this is being made in this laboratory.

The writers are indebted to Dr. S. F. Acree for valuable suggestions and the use of his gas chain apparatus.

REFERENCES

(1) BUNZELL, H. H. 1916 The relationship existing between the oxidase activity of plant juices and their hydrogen-ion concentrations with a note on the cause of oxidase activity in plant tissues. *In* Jour. Biol. Chem., v. 28, p. 315–333.

(2) HAAS, A. R. 1917 The reaction of plant protoplasm. *In* Bot. Gaz., v. 63, p. 232–235.

(3) KAPPEN, H. 1918 Untersuchungen an Wurzelsäften. *In* Landw. Vers. Stat., Bd. 91, p. 1–40.

(4) LOOMIS, N. E., AND ACREE, S. F. 1911 A study of the hydrogen electrode, of the calomel electrode, and of contact potential. *In* Amer. Chem. Jour., v. 46, p. 585–620.

(5) LOOMIS, N. E., AND ACREE, S. F. 1916 The effect of pressure upon the potential of the hydrogen electrode. *In* Jour. Am. Chem. Soc., v. 38, p. 2391–2396.

(6) REED, G. B. 1914 The oxidases of acid tissues. *In* Bot. Gaz., v. 57, p. 528–530.

(7) TRUOG, E. 1918 Soil acidity: I. Its relation to the growth of plants. *In* Soil Sci., v. 5, no. 3, p. 169–195.

(8) WAGNER, R. J. 1916 Wasserstoffionenkonzentration und natürliche immunitat der pflanzen. *In* Centbl. Bakt. (etc.), Abt. 2, Bd. 44, p. 708–719.

THE OXIDATION OF VANILLIN TO VANILLIC ACID BY CERTAIN SOIL BACTERIA

WILLIAM J. ROBBINS AND E. C. LATHROP

Joint contribution from the Alabama Polytechnic Institute and Agricultural Experiment Station, and Soil Fertility Investigations, Bureau of Plant Industry, U. S. Department of Agriculture

Received for publication June 2, 1919

The association of aldehydes with infertility in soils, the isolation of aldehydes from the soil and their harmful effects on plant growth, both in water culture and in the soil, are subjects which in the past few years have engaged the attention of a number of workers, notably Schreiner, Reed, Shorey, Skinner, Sullivan, Noll, Davidson, Fraps, Upson and Powell, and Funchess.[1]

Of particular interest to the question of the relation of aldehydes to soil infertility was the discovery that the aldehyde vanillin persists in some soils but does not persist in others. This appears to be due to the fact that the destruction of vanillin in the soil is chiefly a bacterial process (11). In some soils vanillin-destroying bacteria are present and the soil conditions are favorable for their action on vanillin, while in other soils either the suitable bacteria are absent or conditions are unfavorable for their destructive action on vanillin. A bacterium which uses vanillin with facility as an energy and carbon source, was isolated by one of us from Alabama soil (11).

In the present paper studies are reported which show that in solution cultures consisting of pure inorganic salts and vanillin as the only source of carbon, vanillin is oxidized by this bacterium to vanillic acid and the vanillic acid formed is destroyed. By the use of colorimetric methods the formation of vanillic acid from vanillin and the destruction of the vanillic acid so formed has also been studied.

THE IDENTIFICATION OF VANILLIC ACID AS A PRODUCT OF THE DECOMPOSITION OF VANILLIN BY A SOIL BACTERIUM

Vanillic acid was identified as a product of the decomposition of vanillin by soil bacteria by extracting it with ether from a culture solution containing vanillin, in which a pure culture of vanillin-destroying bacteria had grown.

[1] For a summary and discussion of this work see Reference no. 12.

In a 2-liter flask, 250 cc. of a mineral nutrient solution[2] containing vanillin were placed. The flask and contents were sterilized at 15 pounds pressure for 15 minutes and inoculated with a pure culture of the bacterium referred to above. The flask was incubated at 25–30°C., until the testing of a small portion of its contents by the acid nitrate of mercury reagent described by Estes (2) showed the presence of little or no vanillin while the phenol reagent described by Folin and Denis (3, 4) gave a strong reaction. This usually required from 4 to 5 days' incubation. An ether extract of the contents of the flask at this time yielded a whitish crystalline material consisting almost entirely of vanillic acid.

The identity of this decomposition product with vanillic acid was demonstrated by:

(1) The determination of its melting point and mixed melting point with pure vanillic acid.

(2) Comparison of crystalline form and solubilities with those of vanillic acid.

(3) Color reactions.

(4) Neutralization equivalent.

(5) Methoxyl determination.

(6) Micro-combustion.

The crystalline material obtained by ether extraction as described above was obviously impure, being of a dirty grey color and having the odor of vanillin. The material was dissolved in hot water and filtered. To the filtrate lead acetate solution was added, which in no case caused a precipitate. Ammonia was then added to the solution and the heavy white precipitate which formed was filtered and well washed with distilled water. The precipitate while still moist was ground in a mortar with a little water, suspended in hot water and decomposed with hydrogen sulfide. (It was found that the complete removal of lead from the compound was at times difficult and that the conditions best suited for the removal of lead were large volumes of water and precipitation while hot. In some cases it was found necessary to treat more than once with hydrogen sulfide.) The solution was boiled to expel the excess of hydrogen sulfide, filtered and concentrated on the water bath until crystallization began. The solution was brought to boiling to dissolve all of the crystals which had formed, and the dish containing the hot solution was then chilled and the solution stirred vigorously

[2] The composition of this solution was:

$NaNO_3$	0.1 gm.
K_2HPO_4	0.1 gm.
$MgSO_4$	0.05 gm.
NH_4Cl	0.1 gm.
KCl	0.05 gm.
Vanillin	0.1 gm.
H_2O distilled	200.0 cc.

in order to cause rapid crystallization. The crystals which formed were filtered by suction, taken up in hot water, decolorized with a little pure carbon black, filtered and recrystallized as before. The crystals obtained were masses of long white silky needles which gathered together in clusters.

These crystals were identical in appearance with crystals of vanillic acid purified by passing through the lead salt and crystallized in the manner described.

When the vanillin decomposition product was heated it had a strong odor of vanillin, which is characteristic of vanillic acid. When the decomposition product was heated above its melting point it sublimed in long prisms which were identical in crystalline form with the crystals obtained on subliming pure vanillic acid.

The vanillin decomposition product and pure vanillic acid gave the same color reactions. No color was produced on the addition of a dilute solution of ferric chloride to the dilute solutions tested; on treating a dilute solution of each with a dilute solution of acid mercuric nitrate and heating, a faint pink color was produced in each, and with further application of heat this color faded and a grey precipitate was formed; on treating the dilute solutions with Folin's phosphotungstic phosphomolybdic reagent and then adding sodium carbonate solution in excess, the same shade of blue color developed.

A study of the melting points of the vanillin decomposition product and of vanillic acid showed that the former melted at 210.5–211° (corr.). Vanillic acid melted at 210.5–211°(corr.) and a mixture of the two preparations melted at 210.5°. Tiemann (13) who first prepared vanillic acid found that it melted at 211–212° (uncorr.) and Tiemann and Reimer (16) found that vanillic acid purified by passing through the lead salt melted at 211–212° (uncorr.). Later Tiemann (15) states that the melting point is 207° and not 211° as previously reported. Perkin and Martin (9) found the melting point of vanillic acid to be 205–206°. The melting points given by the text books on organic chemistry are apparently taken from the later determinations.

In taking our melting points we used an apparatus provided with a stirring device similar to that described by Menge (7) . We used 6-inch Anschutz thermometers standardized by the U. S. Bureau of Standards, no stem correction being necessary. Both the crystals of the decomposition product and those of pure vanillic acid melted with slight coloration.

A study of the neutralization equivalent of the vanillin decomposition product and of pure vanillic acid was made. Ostwald (8) has studied the dissociation constant for vanillic acid and gives $K = 0.00298$. The indicator to be used in this case was found to be methyl red, titrating to the full yellow color, although both brom-cresol-purple and phenolphthalein were also tried. The neutralization equivalent determined by the use of indicators was:

	PHENOL-PHTHALEIN	BROM-CRESOL PURPLE	METHYL RED
Vanillic acid	151.2	163.9 160.7	168.7
Vanillin decomposition product		161.3	171.4

The theory for the neutralization equivalent of vanillic acid, $C_8H_8O_4$ is 168. All of the titrations were made with the use of direct color comparisons.

A determination of the methoxyl group in both the vanillin decomposition product and in pure vanillic acid was made by the method of Zeisel (18), the apparatus described by Benedikt and Grüssner (1) being used. The results were as follows:

COMPOUND	METHOXYL FOUND	METHOXYL BY THEORY
	per cent	*per cent*
Vanillic acid	18.76	18.46
Vanillin decomposition product	20.16	

Combustions were made on the vanillin decomposition product and pure vanillic acid by the micro-combustion method developed by Wise[3] (17). An examination of the vanillin decomposition product used for combustion showed that it contained a very small amount of lead. The lead was determined by oxidizing the organic matter with a mixture of sulfuric and nitric acids and weighing the lead as lead sulfate. The results of the combustion of the vanillin decomposition product are given below as found and as corrected for the lead present. No lead could be found in the purified vanillic acid used.

COMPOUND	CARBON	HYDROGEN
	per cent	*per cent*
Pure vanillic acid	56.15	4.88
	56.65	4.67
Vanillin decomposition product	55.20	4.70
Vanillin decomposition product corrected	56.66	4.83
Theory for $C_8H_8O_4$	57.12	4.79

From the analytical data given above the vanillin decomposition product was identified as vanillic acid.

[3] The authors desire to express their thanks to Dr. Wise for his kindness in making these combustions for them.

THE FORMATION AND DESTRUCTION OF VANILLIC ACID

In solution cultures containing nutrient mineral salts and vanillin and inoculated with the bacterium referred to above the vanillin is oxidized to vanillic acid, which accumulates in considerable amount in the solution and is then destroyed. These facts were developed in attempts to explain the results which were obtained in using the phenol reagent to determine the vanillin content of solution cultures which had been inoculated with the vanillin-destroying bacterium. When the vanillin content of mineral nutrient solution cultures which had been inoculated with the vanillin-destroying bacterium was determined by the phenol reagent it was found that the vanillin content apparently rose enormously in the early stages of digestion but later decreased to zero. When the Estes reagent was used for making the determination the vanillin content showed no such apparent increase but uniformly decreased to the zero point.

The details of an experiment[4] will illustrate these statements.

A number of cultures were prepared containing 40 cc. of mineral nutrient solution[5] in a 150-cc. Erlenmeyer flask. The reaction of the solution was made −2, Fuller's scale, with NaOH. The flasks were sterilized at 15 pounds pressure for 15 minutes and after sterilization 10 cc. of a sterile solution of commercial vanillin were added to each flask. All flasks, save some checks, were inoculated with three drops of a suspension of a pure culture of the vanillin-destroying bacterium. Two cultures and a check flask were analyzed daily with both the acid nitrate of mercury and the phenol reagent.

In making the color determinations with the Estes reagent 1 cc. of the reagent was added to 5 cc. of the culture solution. The mixture was diluted with water and boiled for 5 minutes on a hot plate. The solution was then filtered and made up to 50 cc. with distilled water. The color developed in a similar way by 5 cc. of a solution of 200 parts per million of vanillin was used as a standard. The phenol reagent was used as described by Folin and Denis with the clarification by means of lead acetate omitted. No considerable degree of accuracy is claimed for these determinations, as no attempt was made to vary the concentration of the standard with the variation in the apparent amount of vanillin present in the cultures. In the temporary absence of a colorimeter the comparisons were made with colorimeter tubes of equal diameter.

The results are presented in table 1 and figure 1. In the first column of the table the time in days from the day of inoculation is given. The

[4] The assistance of A. B. Massey in performing this experiment is acknowledged.

[5] The mineral composition of this solution was:

K_2SO_4	0.037 gm.
$NaNO_3$	0.100 gm.
$CaH_4(PO_4)_2.H_2O$	0.071 gm.
Water (distilled)	3000 cc.

figures in the second column are the vanillin contents in parts per million as determined by the Estes reagent. Column 3 represents the values obtained with the phenol reagent expressed in parts per million of vanillin. In the figure the time in days is given on the abscissa and the vanillin content of the cultures in parts per million as determined by the Estes reagent and by the phenol reagent on the ordinate.

From the data in table 1 and the curves in figure 1 it can be noted that the vanillin as determined by the Estes reagent uniformly decreased, sharply at first, more slowly later, until no vanillin was found on the eighth day from the day of inoculation. Using the phenol reagent to determine the vanillin, however, the vanillin content rapidly increased until on the third

TABLE 1

Data showing formation and destruction of vanillic acid

TIME FROM INOCULATION	VANILLIN BY ACID NITRATE OF MERCURY	VALUES WITH PHENOL REAGENT EXPRESSED AS P.P.M. OF VANILLIN
	p.p.m.	*p.p.m.*
Average of checks	249.0	285.0
1 day	257.0	282.0
2 days	177.0	343.0
3 days	56.0	504.0
4 days	23.0	482.0
5 days	22.0	270.0
6 days	26.0	151.0
7 days	10.0	85.0
8 days	0.0	80.0
9 days		17.0

day apparently almost twice as much vanillin was present as was originally added to the solutions. From that point the vanillin decreased until by the ninth day it was practically exhausted.

An examination of the color produced by vanillin and vanillic acid with Estes' reagent and by vanillin and vanillic acid with the phenol reagent explained the anomalous results secured with the phenol reagent.

Pure vanillic acid was prepared by the method already described and pure vanillin was prepared by the following method:

Commercial vanillin was dissolved in ether and the ether extract was shaken out with a freshly prepared sodium bisulfite solution. The sodium bisulfite solution was then acidified with dilute sulfuric acid and as much as possible of the sulfur dioxide was removed by violent shaking of the flask. The acidified solution was then shaken out with ether which had been previously purified from other aldehydes by shaking with a little saturated sodium bisulfite solution. The ether solution was concentrated until all of the ether had evaporated and the molten mass of vanillin was dissolved in boiling water. The solution was filtered while hot and the vanillin crystallized by placing

the beaker in chipped ice. The crystals were filtered off in a Buchner funnel and recrystallized twice more. The pure vanillin was then dried on a porous plate in a desiccator over calcium chloride. It was found to melt at 80.5–81° (corr.).

Experiments with Estes' reagent. When a solution of vanillin was heated with Estes' reagent a clear pink-colored solution resulted. Vanillic acid, however, first produced a pink color which on further heating faded and in

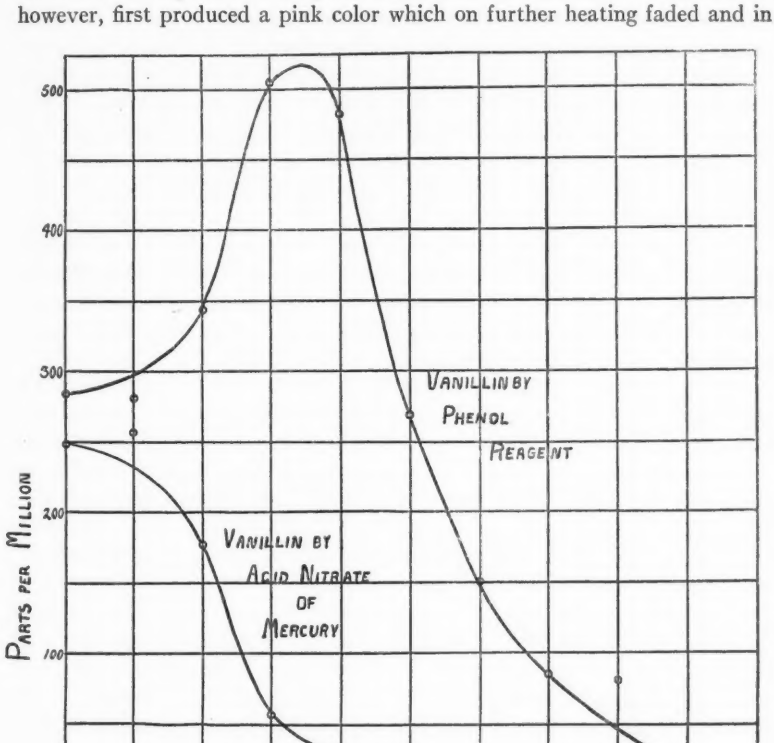

Fig. 1. Diagram Showing Formation and Destruction of Vanillic Acid

its place an easily filtered gray or black precipitate was formed. The amount of pink color remaining in the vanillic acid solution depended on the amount of heating. The color produced by heating 10 cc. of 100 parts per million of vanillic acid and 1.5 cc. of Estes' reagent on the steam bath for 20 minutes was about 3 per cent of that produced by the same concentration of vanillin. When the solutions were boiled for five minutes on a hot plate and allowed

to cool the color produced by the vanillic acid was too weak to be read by the Schreiner colorimeter.

Experiments with Folin's reagent. With Folin's reagent both vanillin and vanillic acid were found to produce a deep blue color, the color produced by the vanillic acid being of a slightly different shade of blue and considerably deeper. Thus 5 cc. of 200 parts per million of vanillin were treated with 5 cc. of the phenol reagent, allowed to stand 10 minutes, and made up to 50 cc. with saturated sodium carbonate solution. After standing 15 minutes the solution was filtered and made up to 100 cc. with distilled water. Comparison in a Schreiner colorimeter of the color produced with that developed in a solution of vanillic acid of 200 parts per million treated in the same way showed that the color produced by the vanillic acid was about 1.8 times as strong as that developed by the vanillin. Using solutions of 100 parts per million, a factor of about 1.5 was obtained.

Determinations of vanillin and vanillic acid in mixtures. Since vanillin and vanillic acid both produced a blue color with the phenol reagent and vanillin alone reacts with the Estes reagent to any considerable extent it was thought that a method could be evolved for determining vanillin and vanillic acid in mixtures by using the two reagents. The method proposed consisted in making a determination colorimetrically of the solution containing vanillin and vanillic acid, first with the acid nitrate of mercury reagent described by Estes, using a suitable solution of vanillin as a standard; and, second, with the phenol reagent described by Folin and Denis, using a suitable solution of vanillin as a standard. The results with the Estes reagent should give the quantity of vanillin present. The difference between the results obtained with the Folin's reagent and those obtained with the Estes reagent divided by a suitable factor should equal the vanillic acid.

It was found that by using a standard of 100 parts per million vanillin could be determined by the Estes method in concentrations of 25 to 400 parts per million in the presence of from 50 to 800 parts per million of vanillic acid. On the other hand, vanillic acid could not be accurately estimated within such a wide range of concentration. With a strong concentration of vanillin, 250 to 400 parts per million, and a weak concentration of vanillic acid, 25 to 50 parts per million, the results with the phenol reagent were far too low. With other mixtures the factor necessary was found to vary from about 0.7 to about 2.0. Thus with a standard of 200 parts per million of vanillin a mixture containing 175 parts per million of vanillin and 100 parts per million of vanillic acid gave a result of 296 parts per million with the phenol reagent. Since the mixture contained 175 parts per million of vanillin, 121 parts per million of the result were due to the vanillic acid. Dividing this by the actual amount of vanillic acid present, the factor of 1.21 was obtained. In a mixture of 25 parts per million of vanillin and 200 parts per million of vanillic acid the factor was 1.91. With a standard of 100 parts per million the factor found for a mixture of 300 parts per million of vanillin and 100 parts per million of vanillic acid was 0.76.

The results, therefore, indicated that without a further and more complete study vanillic acid could not be accurately determined quantitatively by the method proposed in mixtures of vanillin and vanillic acid.

Sufficient information was secured, however, to permit an interpretation of the results obtained in the bacteriological experiment mentioned above. The results with the Estes reagent in column 2 of table 1 indicate the amount of vanillin present. The apparent increase in the amount of vanillin present as determined by the phenol reagent is due to the formation of vanillic acid. Since the vanillic acid formed produces more color with the phenol reagent than the vanillin which it replaced, the color developed was greater than that produced by the original amount of vanillin. The decrease in the amount of vanillin as determined by the phenol reagent which occurred after the third or fourth day is evidently due to the fact that the vanillin had been oxidized for the most part to vanillic acid, and due to its destruction the amount of vanillic acid present had begun to decrease. In other words, in the early stages of digestion vanillic acid accumulated in the culture solution, the vanillin being oxidized more rapidly than the vanillic acid was destroyed, and the accumulated vanillic acid was then destroyed.

A rough approximation from the data in table 1 can be made of the amount of vanillic acid present on the third day. In a mixture of 50 parts per million of vanillin and 225 parts per million of vanillic acid the color developed with the phenol reagent compared with that developed by a standard of 200 parts per million of vanillin was 400 parts per million. Since there were present 50 parts per million of vanillin, the vanillic acid evidently produced 350 parts per million of the color. Dividing this figure by the amount of vanillic acid present gives us the factor of 1.55. On the third day from the day of inoculation 56 parts per million of vanillin were found by the Estes reagent to be present in the culture solution. With the phenol reagent the figure was 504 parts per million. Subtracting from this figure the value found with the Estes reagent and dividing by the above factor, we have a value for the vanillic acid of 288 parts per million. This figure is greater by some 70 parts per million than is possible from the amount of vanillin which had disappeared from the culture solution. This is probably due to inaccuracies in the original determination and to the fact that the factor used was determined for a mixture which only approximated the proportions of vanillin and vanillic acid actually present in the culture solution. The results indicate, however, that little of the vanillic acid formed had been destroyed by the third day.

What products in addition to vanillic acid are formed by the action of the bacteria studied on vanillin it is impossible to state at present. The fact that a sterile solution of vanillin and mineral nutrient salts inoculated with a pure culture of the vanillin-destroying bacteria will eventually give no test with the phenol reagent of Folin and Denis indicates that the final products of the action of this organism on vanillin probably no longer possess a phenolic nature.

That the final products are not toxic to wheat has been demonstrated in an earlier publication (11).

From the chemical standpoint the oxidation of vanillin to vanillic acid by microorganisms is most interesting. Tiemann (13) attempted to prepare vanillic acid from vanillin by oxidizing the latter with many oxidizing reagents but all of these attempts were unsuccessful since he either was not able to change the vanillin at all or the oxidation proceeded too far with the formation of resinous bodies. Tiemann (14) observed, however, that if vanillin were allowed to stand in moist condition in contact with the air, it was slowly oxidized to vanillic acid. Preusse (10) observed that on feeding vanillin to dogs vanillic acid in small amount was excreted in the urine. He attempted to oxidize vanillin to guaiacol by pancreatic digestion but failed. Kotake (6) fed vanillin to dogs and found that it was oxidized to vanillic acid and excreted in the urine in the form of the paired glycuronic vanillate which he separated in the form of the barium salt. Harder (5) observed that a standard solution of vanillin in water on standing for some time gave inaccurate results with the phenol reagent, probably due to the formation of vanillic acid, and he suggested a standard prepared in approximately 20 per cent alcohol.

The demonstration that the destruction of vanillin is a biological oxidative process is of importance from the standpoint of soil fertility[6] because it indicates that the elimination of this compound in the soil is influenced by the factors which affect the supply of oxygen to the soil.

SUMMARY

1. Vanillic acid was isolated from solution cultures containing nutrient mineral salts and vanillin, and inoculated with a pure culture of a soil bacterium.

2. The melting point of vanillic acid is 210.5–211°C. (corr.).

3. Both vanillin and vanillic acid produce a blue color with the phenol reagent of Folin and Denis. Vanillic acid gives only a faint color with Estes' acid nitrate of mercury.

4. In solution cultures containing mineral nutrient salts and vanillin and inoculated with a pure culture of the vanillin-destroying bacteria, isolated from the soil, the vanillin is oxidized to vanillic acid. The vanillic acid at first accumulates in the culture medium but is later destroyed.

[6] We have found by the use of Estes' acid nitrate of mercury and the phenol reagent that vanillin is oxidized to vanillic acid in the soil as well as in solution cultures.

REFERENCES

(1) BENEDIKT, R., AND GRÜSNER, A. 1889 Zur quantitativen Bestimmung von Methoxyl. *In* Chem. Ztg., Bd. 13, No. 53, p. 872–873.

(2) ESTES, D. 1917 A new quantitative test and colorimetric method for the estimation of vanillin. *In* Jour. Indus. Engin. Chem., v. 9, no. 2, p. 142–144.

(3) FOLIN, O., AND DENIS, W. 1912. On phosphotungstic and phosphomolybdic compounds as color reagents. *In* Jour. Biol. Chem., v. 12, p. 239–243.

(4) FOLIN, O., AND DENIS, W. 1912 A new colorimetric method for the determination of vanillin in flavoring extracts. *In* Jour. Indus. Engin. Chem., v. 4, no. 9, p. 670–672.

(5) HARDER, O. E. 1913 Note on the standard vanillin solution for the colorimetric method for the determination of vanillin in flavoring extracts. *In* Jour. Indus. Engin. Chem., v. 5, no. 7, p. 619.

(6) KOTAKE, Y. 1905 Ueber das Schicksal des Vanillins im Tierkörper. *In* Ztschr. Phys. Chem., Bd. 45, p. 320–325.

(7) MENGE, G. A. 1910 A study of melting-point determinations. U. S. Treas. Dept. Hyg. Lab. Bul., 70, p. 1–101.

(8) OSTWALD, W. 1889 Ueber die Affinitätsgrössen organischer Säuren und ihre Beziehungen zur Zusammensetzung und Konstitution derselben. *In* Ztschr. Phys. Chem. Bd. 3, p. 241–288.

(9) PERKIN, A. G., AND MARTIN, H. W. 1897 LXXVII Rhamnazin. *In* Jour. Chem. Soc. London, v. 71, p. 818–822.

(10) PREUSSE, C. 1880 Ueber das Verhalten des Vanillins in Thierkörper. *In* Ztschr. Phys. Chem., Bd., 4, p. 209–213.

(11) ROBBINS, W. J. 1917 The cause of the disappearance of cumarin, vanillin, pyridine, and quinoline in the soil. Ala. Agr. Exp. Sta. Bul. 195, p. 49–64.

(12) SKINNER, J. J. 1918 A scientific study of a new class of soil constituents unfavorable to crops, their occurrence, properties, and elimination in practical agriculture. *In* Jour. Franklin Inst., v. 186, no. 2, p. 165–186; no. 3, p. 289–316; no. 4, p. 449–480; no. 5, p. 547–584; no. 6, p. 723–741.

(13) TIEMANN, F. 1875 Ueber Vanillinsäure. *In* Ber. Deut. Chem. Gesell., Bd. 8. p. 509–515.

(14) TIEMANN, F. 1875 Ueber eine synthetische Bildungsweise des Vanillins und einige Reduction des Letzteren darstellbare Verbindungen (Hydrovanilloin und Vanillylalkohol). *In* Ber. Deut. Chem. Gesell., Bd. 8, p. 1123–1127.

(15) TIEMANN, F. 1896 Ueber die Coniferyl-und Vanillinreihe angehörigen Verbindungen. *In* Ber. Deut. Chem. Gesell, Bd., 9, p. 409–423.

(16) TIEMANN, F., AND REIMER, C. 1875 Ueber Zuckervanillinsäure, ein neues Glucosid. *In* Ber. Deut. Chem. Gesell., Bd. 8, p. 515–518.

(17) WISE, L. E. 1917 A simplified micro-combustion method for the determination of carbon and hydrogen. *In* Jour. Amer. Chem. Soc., v. 39, p. 2055–2068.

(18) ZEISEL, S. 1885 Ueber ein Verfahren zum quantitativen Nachweise von Methoxyl. *In* Monatsh. Chem., Bd. 6, p. 989–996.

THE ELECTROMETRIC TITRATION OF PLANT JUICES[1]

A. R. C. HAAS

From the Department of Soils and Agricultural Bacteriology, University of Wisconsin[2]

Received for publication August 19, 1919

The reaction of plant protoplasm is of fundamental importance in studies upon plant metabolism (1, 3, 8). Although life processes are affected primarily by the actual acidity[3] of the protoplasm; nevertheless, the total acidity may become of importance in various ways.

The actual acidity of plant juices may be determined by means of electrometric or colorimetric methods (2, 7, 9). The total acidity is usually determined by titration with standard alkali, using an indicator like phenolphthalein or litmus. This has yielded important data regarding the metabolism of succulent plants (4, 5). However, it has been observed that in some cases the natural coloring matter in plant juices makes the use of indicators difficult and the results doubtful.

In this work the desirability of having a method which is not dependent on indicators is clearly evident. It is important also to know not only the actual acidity of the extracted (6) juice, but also the actual acidity after any addition of alkali, together with the value for the total acidity corresponding to this actual acidity. Fortunately, such a method is available, but apparently has not been employed by investigators working upon plant juices. The method is that of the gas-chain, essentially of the form described by Hildebrand (8).

An inexpensive and simplified gas-chain of this type was set up. A capillary electrometer, with an improvised horizontal microscope, served as a zero instrument. A Weston voltmeter, reading directly to 0.01 volt and capable of being estimated to 0.001 volt, was used to measure the electromotive force. Several dry cells arranged in parallel and attached to a Wheatstone slidewire bridge, furnished the voltage required. The calomel cell and the electrode employed were similar to those described by Bovie (8), except that a narrow sheet of platinum was used as the electrode instead of a platinum wire. Hydrogen was obtained from a Kipp generator containing arsenic-free zinc and C.P. hydrochloric acid. The hydrogen was purified by

[1] A preliminary paper on a phase of the study of the relation of soil acidity to plants and bacteria.

[2] Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

[3] By actual acidity is meant the quantity of dissociated acids present; whereas, the total acidity includes both the dissociated and the undissociated acids.

passing it through wash bottles containing successively potassium permanganate, alkaline pyrogallol (2 bottles), and alkali. The last wash-bottle was used primarily to observe and regulate the rate of flow of hydrogen. By attaching a pressure bottle to the top of the Kipp generator it was possible to secure a uniform flow of hydrogen.

The titration vessel consisted of a glass test tube (about 3 by 10 cm.) fitted with a ½-inch thick rubber stopper. The calomel electrode and titration vessel were placed in an electrically heated water bath regulated at 25°C. The capillary connecting tube, containing KCl, and the electrode, were inserted through holes in the stopper. Two other holes were also cut in the rubber stopper—one for insertion of the burette tip (which should be long enough to extend to within about 1 inch of the surface of the plant juice), and the other a small opening for the exit of gas. Ordinarily a Bunsen valve can be used for the exit of gas, but frequently the frothing of the plant juice required the use of a glass tube blown out at one end into a small bulb punctured with fine holes. These holes can be made readily by means of a hot platinum wire. This tube was inserted through the rubber stopper, leaving the bulb in the upper portion of the titration vessel. By carefully regulating the flow of hydrogen, losses by frothing do not occur. The results obtained with the apparatus just described were frequently compared with those obtained with another gas chain in this laboratory operated by C. B. Clevenger. This was equipped with a Leeds and Northrup potentiometer, a sensitive galvanometer and electrolytically prepared hydrogen. Determinations of actual acidity, when carried out on both pieces of apparatus, were seldom more than 0.05 P_H apart and frequently differed by only 0.01 P_H. The voltmeter apparatus, as described, was found so simple, accurate and extremely rapid, that it was chosen for the titrations of the plant juices.

The juice of the plants was usually secured as follows: The tissues were thoroughly macerated and placed on a clean linen cloth; the juice was then expressed by turning the ends of the cloth in opposite directions.

Maceration by the above method was more desirable than with a mortar and pestle. The linen cloth was more convenient than a Witt laboratory press for expressing the juice. The cloth was thoroughly washed with pure water after each use. In the case of many plants it was found impossible to secure colorless solutions even by filtering, which was consequently omitted. The juice was usually centrifuged for 10 minutes to remove coarse suspended material. On standing, the acidity of some plant juices changed slightly and hence in some cases centrifuging was omitted in order to expedite the titration.

By means of a pipette a measured quantity of the juice was than transferred to the titration tube. The calomel cell connections were made and the electrode was then placed in the titration tube. It was found advantageous to have the electrode tube permanently held at the desired height in

the stopper of the titration tube. The hydrogen was then bubbled through the plant juices, and the actual acidity P_H, was determined in the usual manner. The burette tip was then inserted through the rubber stopper and a measured quantity of alkali added. After thoroughly mixing the solution by means of the bubbling hydrogen, a second reading of the actual acidity P_H was made. It was found that considerable time could be saved by bubbling hydrogen at first quite rapidly through the solution for a few moments and then very slowly; so that the bubbling of the gas was scarcely perceptible. Equilibrium was then rapidly attained. Successive additions of alkali were similarly made and the actual acidity P_H corresponding to each new equilibrium was obtained.

As examples of such determinations [4] the data for soybeans and rhubarb are given. Medium yellow soybeans were planted April 17 in yellow sand to which had been added $CaCO_3$, $(NH_4)_2SO_4$, KH_2PO_4, $Fe_3(PO_4)_2$, $MgSO_4$, and nodule-bacteria. The tops of the plants were cut May 28, the juice was extracted and centrifuged for 15 minutes and finally 10 cc. of the juice was run into the titration tube. The data obtained are as follows:

ACTUAL ACIDITY	0.0684 N NaOH ADDED
P_H	*cc.*
5.85	0
6.04	1.0
6.28	2.0
6.49	3.0
6.70	4.0
6.88	5.0
7.05	6.0
7.29	7.0
7.72	8.0
8.05	9.0
8.22	10.0
8.54	11.0
8.82	12.0
9.20	13.0

A rhubarb stalk (with leafblade removed) was taken directly from the field and the juice extracted and centrifuged for 10 minutes. The values obtained when 8 cc. of juice was used, are as follows:

[4] These examples in which soybean tops are compared with rhubarb stalks are not intended for critical comparison; since the writer has found, as will be indicated in subsequent papers, that the acidity of the leaves, stems and roots of the same plant may differ quite markedly in their actual and total acidities.

ACTUAL ACIDITY	0.0684 N NaOH ADDED
P_H	cc.
3.36	0
3.62	1.85
3.83	4.85
4.35	8.95
4.79	12.95
5.09	14.85
8.41	18.95
9.27	19.35
10.00	19.85
10.63	20.95

Curves are given in figure 1, showing the actual acidity PH against the cubic centimeters of standard alkali added.

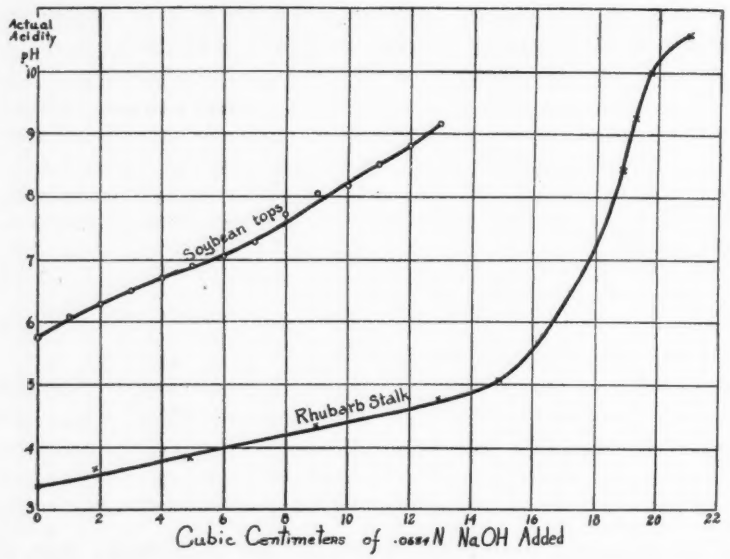

FIG. 1. CURVES SHOWING THE RELATION OF ACTUAL ACIDITY PH WITH THE AMOUNT OF STANDARD ALKALI ADDED, FOR THE SOYBEAN AND RHUBARB

It is evident that if the PH values at which indicators change color are known, it is possible to tell from the curves the quantity of alkali required to bring the plant juice to the turning point for any indicator. For example, in the curve for soybean tops, since the turning point of litmus is at PH 6.83, it is evident that 4.9 cc. of 0.0684 N NaOH must be added to the 10 cc. of juice to reach the turning point for litmus. If we choose phenolphthalein as

the indicator (with its color change at PH 8.3) it is seen from the curve that 10.3 cc. of 0.0684 N NaOH are required in order to bring the 10 cc. of juice to the turning point of phenolphthalein. By reducing the quantity of alkali added each time, it is possible to define the curves more accurately. It is obvious that the curves show the presence of greater quantities of buffer substances in the juice of the rhubarb than in the juice of soybean tops. The actual acidity of rhubarb juice is seen to be much greater than that of soybean tops. The curve for rhubarb rises very gradually at first, but once the buffer substances have been used up, the curve becomes quite abrupt.

Obviously, by titrating with acid instead of alkali, it is possible to prolong the lower ends of the curves. These methods of attack open a fertile field of study. It includes the determination of the buffer processes, the acid and the alkali reserve and the actual and total acidities of plant juices. Furthermore, the method makes possible a study of some of the relations of the nutrients in the soil upon the actual and total acidities of plant juices and the relations of the symbiotic bacteria to these.

Detailed studies of these and other phases of the reaction of plant juices are in progress.

REFERENCES

(1) BUNZELL, H. H. 1916 The relationship existing between the oxidase activity of plant juices and their hydrogen-ion concentration, etc. *In* Jour. Biol. Chem., v. 28, no. 1, p. 315–333.

(2) HEMPEL, JENNY 1917 Buffer processes in the metabolism of succulent plants. *In* Compt. Rend. Lab. Carlsberg, t. 13, no. 1, p. 130.

(3) LLOYD, F. E. 1918 The effect of acids and alkalis on the growth of the protoplasm in pollen tubes. *In* Mem. Torrey Bot. Club, v. 17, p. 84–89.

(4) MACDOUGAL, D. T., RICHARDS, H. M., AND SPOEHR, H. A. 1919 Basis of succulence in plants. *In* Bot. Gaz., v. 67, p. 405.

(5) RICHARDS, H. M. 1915 Acidity and gas interchange in cacti. *In* Carnegie Inst. Wash. Pub. 209, p. 107.

(6) RICHARDS, H. M. 1918 Determination of acidity in plant tissues. *In* Mem. Torrey Bot. Club, v. 17, p. 241–245.

(7) ROSE, R. C. 1919 After-ripening and germination of seeds *Tilia*, *Sambucus*, and *Rubus*. *In* Bot. Gaz., v. 67, p. 281.

(8) SCHMIDT, C. L. A., AND HOAGLAND, D. R. 1919. Table of P_H, H^+, and OH^- values corresponding to electromotive forces determined in hydrogen electrode measurements, with a bibliography. *In* Univ. Cal. Pub. Physiol., v. 5, p. 44–69.

(9) THOMPSON, F. 1918 Report of the chemist: Fruit juices. *In* Del. Agr. Exp. Bul. 119 (Ann. Rpt. 1917), p. 18.

INDEX

Acidity of plant juice, as related to soil acidity, 469–474
Acidity of soils, 197–200
Agar, effect of, on the growth of wheat seedlings, 205–206
Alkali Salts in Soils, The Inhibition by Stable Manure of the Injurious Effects of (paper), C. B. Lipman and W. F. Gericke, 105–120.
  discussion, 118–120
  plan of the experiment, 106
  sodium carbonate, 116–118
  sodium chloride, 106–111
  sodium sulfate, 111–115
Alkalinity, changes in the soil, 268–269
Aluminum, iron, manganese, affected by sulfofication and nitrification, 194
Aluminum hydroxide, effect of, on the growth of wheat seedlings, 214
Ames, J. W., and Boltz, G. E. (paper), Effect of Sulfofication and Nitrification on Potassium and other Soil Constituents, 183–195
Ammonia, Carbon Dioxide, and Nitrate, Parallel Formation of, in Soil (paper), P. L. Gainey, 293–311
Ammonia-fixing Capacity of Calcium Sulfate, The (paper), F. E. Bear and A. C. Workman, 283–291
Ammonia, production of, in heated soils, 45–59
Ammonium sulfate, relative value of, as a fertilizer, 122–126

Bacillus radicicola, The Longevity of, on Legume Seeds (paper), C. R. Fellers, 217–232
  discussion of results, 225–230
  media used in the work, 219–222
  procedure, 223–225
  references, 231–232
  summary, 230–231
  treatment of seed, 218–219
Bacteria stimulated by salts, 137–141
Bacteria, solvent action of, 141–144
Bear, F. E., and Workman, A. C. (paper), The Ammonia-fixing Capacity of Calcium Sulfate, 283–291
Boltz, G. E., Ames, J. W., and (paper), Effect of Sulfofication and Nitrification on Potassium and other Soil Constituents, 183–195
Botanical Composition of a Permanent Pasture as Influenced by Fertilizers of Different Compositions (paper), J. J. Skinner and C. F. Noll, 161–179
  fertilizers, influence of, on yield, 164–167
  discussion and summary, 172–175
  plan of the experiment, 162–164
  triangles, north and south, 168–172

Calcium and magnesium, solubility of, affected by solfofication and nitrification, 192–194
Calcium carbonate, effect of, on the solubility of plant-food, 127–128
Calcium hypochloride used in sterilization of seeds, 219
Calcium sulfate—
  as a fertilizer, 129
  the ammonia-fixing capacity of, 283–291
Carbon, total, in the soil, 261–262
Carbon Dioxide, Ammonia and Nitrate, Parallel Formation of, in Soil (paper), P. L. Gainey, 293–311
  aeration, effect of, 303–309
  discussion and summary, 309–311
  methods, 293–296
  moisture content, effect of, 297–302
  references, 311
Carbon dioxide, effect of, on soluble plant-food in soil, 136
Carbon dioxide in the soil atmosphere, 261
Capillarity, The Movement of Moisture in Soil by (paper), W. Gardner, 313–317
Capillary Moisture-holding Capacity (paper) W. Gardner, 319–324
Carter, E. G., Greaves, J. E., and (paper), The Action of Some Common Soil Amendments, 121–160

Colloidal Substances, The Effect of Certain, on the Growth of Wheat Seedlings (paper), D. W. Jennings, 201–215
agar cultures, 205–206
aluminum hydroxide cultures, 214
ferric hydroxide cultures, 212–213
plan of the investigation, 203–204
references, 215
sand cultures, 209–212
silica cultures, 207–209
summary, 215
Cylinder Experiments, The Abnormality of Soils in Field-placed, (paper), C. A. Mooers, 247–251

Distillation (dry) of soils, 63–65

Fellers, C. R. (paper), The Longevity of *Bacillus radicicola* on Legume Seeds, 217–232
Ferric hydroxide, effect of, on the growth of wheat seedlings, 212–213
Fertilizers, influence of, on yield and botanical composition of the vegetation of a permanent pasture, 164–168
Formalin used in sterilization of seeds, 218–219
Fred, E. B.—
and Graul, E. J. (paper), Effect of Inoculation and Lime on the Yield and on the Amount of Nitrogen in Soybeans on Acid Soil, 455–467
Haas, A. R. C., and (paper), The Effect of Soybean Germination upon the Growth of its Nodule-forming Bacteria, 237–245
Freezing-point Method, The Comparative Rate of Formation of Soluble Material in Cropped and Virgin Soils as Measured by, (paper), C. E. Millar, 253–257
Fungi, development of, in heated soils, 36–39

Gainey, P. L. (paper) Parallel Formation of Carbon Dioxide, Ammonia and Nitrate in Soil, 293–311
Gardner, W. (paper)—
Capillary Moisture-holding Capacity, 319–324
The Movement of Moisture in Soil by Capillarity, 313–317
Gericke, W. F., Lipman, C. B., and (paper), The Inhibition by Stable Manure of the Injurious Effects of Alkali Salts in Soils, 105–120
Graul, E. J., Fred, E. B., and (paper), Effect of Inoculation and Lime on the Yield and on the Amount of Nitrogen in Soybeans on Acid Soil, 455–467
Greaves, J. E., and Carter, E. G. (paper), The Action of Some Common Soil Amendments, 121–160

Haas, A. R. C.—
(paper), The Electrometric Titration of Plant Juices, 487–491
and Fred, E. B. (paper), The Effect of Soybean Germination upon the Growth of its Nodule-forming Bacteria, 237–245
Hartwell, B. L., Pember, F. R., and Howard, L. P. (paper), Lime Requirements as Determined by the Plant and by the Chemist, 279–282
Hibbard, P. L. (paper), Changes in Composition of the Soil and of the Water Extract of the Soil, Following Addition of Manure, 259–272
Hoagland, D. R., Sharp, L. T., and (paper), Note on Recent Work Concerning Acid Soils, 197–200
Howard, L. P., Hartwell, B. L., Pember, F. R., and (paper), Lime Requirements as Determined by the Plant and by the Chemist, 279–282
Hydrogen peroxide used in sterilization of seeds, 219

Inoculation, Effect of, and Lime on the Yield and on the Amount of Nitrogen in Soybeans on Acid Soil (paper), E. B. Fred and E. J. Graul, 455–467
Iron sulfate, as a fertilizer, 130–131

Jennings, D. S. (paper), The Effect of Certain Colloidal Substances on the Growth of Wheat Seedlings, 201–215
Johnson, J. (paper), The Influence of Heated Soils on Seed Germination and Plant Growth, 1–103

Lathrop, E. C., Robbins, W. J., and (paper), The Oxidation of Vanillin to Vanillic Acid by Certain Soil Bacteria, 475–485
Leaching, involved in comparison of residual calcium carbonate, 380–385
Legume Seeds, The Longevity of *Bacillus radicicola* on (paper), C. R. Fellers, 217–232

Lime—
effect of—
on phosphorus, 128–129
on yield and amount of nitrogen in soybeans on acid soil, 455–467
Requirements as Determined by the Plant and by the Chemist (paper), B. L. Hartwell, F. R. Pember, and L. P. Howard, 279–282
The Carbonation of Burnt Lime in Soils (paper), W. H. MacIntire, 325–453
burnt lime, chemical action upon soil organic matter, 423–436
carbon dioxide, source of, in combining with $Ca(OH)_2$ lying upon the soil surface, 408–413
chemistry of carbonation of burnt lime, 331–342
cylinder field studies, 349–366
historical, 327–331
introduction, 325–327
leaching involved in comparison of residual calcium carbonate, 380–385
lime, hydrated, persistence in soils, 343–346
lysimeter field tanks, carbonation studies in, 370–385
methods of analysis used in soil studies, 342–349
pot studies in the field, 366–370
references, 443–446
speed of carbonate reaction, 414–423
summary and conclusions, 436–442
tumbler experiments, 393–408
Limestone, The Comparative Value of Various Forms of, (paper), R. Stewart and F. A. Wyatt, 273–278
Lipman, C. B., and Gericke, W. F. (paper), The Inhibition by Stable Manure of the Injurious Effects of Alkali Salts in Soils, 105–120
Lipman, J. G. (editorial), Adjusting the Soil Reaction to the Crop, 181
Lysimeter field tanks, carbonation studies in, 370–393

Manganese compounds, as fertilizers, 132–133
Manure—
changes in composition due to rotting, 268
The Inhibition by Stable, of the Injurious Effects of Alkali Salts in Soils (paper), C. B. Lipman and W. F. Gericke, 105–120
MacIntire, W. H. (paper), The Carbonation of Burnt Lime in Soils, 325–453
McMiller, P. R. (paper), Some Notes on the Cause of Unproductivity of "Raw" Subsoils in Humid Regions, 233–236
Meacham, M. R., Truog, E., and (paper), Soil Acidity: II. Its Relation to the Acidity of the Plant Juice, 469–474
Mercuric bichloride, use in sterilization of seeds, 219
Microorganisms, conditions favoring development of heated soils, 74–76
Millar, C. E. (paper), The Comparative Rate of Formation of Soluble Material in Cropped and Virgin Soils, as Measured by the Freezing-point Method, 253–257
Mooers, C. A. (paper), The Abnormality of Soils in Field-placed Cylinder Experiments, 247–251
Moisture in Soil, The movement of, by Capillarity (paper), W. Gardner, 313–317
Moisture-holding Capacity, Capillary (paper), W. Gardner, 319–324

Nitrate—
Carbon Dioxide, Ammonia, and, Parallel Formation of, in Soil (paper), P. L. Gainey, 293–311
changes in the soil, 267–268
total, in the soil, 262, 264
Nodule-forming Bacteria, The Effect of Soybean Germination upon the Growth of (paper), A. R. C. Haas and E. B. Fred, 237–245
Noll, C. F., Skinner, J. J., and (paper), Botanical Composition of a Permanent Pasture as influenced by Fertilizers of Different Compositions, 161–179

Phosphorus, acted upon by salts and bacteria, 145–151
Pember, F. R., Howard, L. P., Hartwell, B. L., and (paper), Lime Requirements as Determined by the Plant and by the Chemist, 279–282
Plant growth on heated soils, 23–36
Plant-food, fixation of, by soil, 265–266
increase of, in soil, 269–270
Plant juice, acidity of, as related to soil acidity, 469–474

Plant Juices, The Electro-metric Titration of (paper), A. R. C. Haas, 487–491
Potassium, solubility of, affected by sulfofication and nitrification, 187–192

Robbins, W. J., and Lathrop, E. C. (paper), The Oxidation of Vanillin to Vanillic Acid by Certain Soil Bacteria, 475–485

Saline solution, effect of, on phosphates, 133–136
Sand, effect of, on the growth of wheat seedlings, 209–212
Seed germination on heated soils, 8–22
Sharp, L. T., and Hoagland, D. R. (paper), Note on Recent Work Concerning Acid Soils, 197–200
Silica, effect of, on the growth of wheat seedlings, 207–209
Skinner, J. J., and Noll, C. F. (paper), Botanical Composition of a Permanent Pasture as Influenced by Fertilizers of Different Compositions, 161–179
Sodium carbonate, inhibition of its injurious effect on soils by stable manure, 116–118
Sodium chloride—
as a fertilizer, 126–127
inhibition of its injurious effect on soils by stable manure, 111–115
Soil—
Acidity: II. Its Relation to the Acidity of the Plant Juice (paper), E. Truog and M. R. Meacham, 469–474
Amendments, The Action of Some Common, (paper), J. E. Greaves and E. G. Carter, 121–160
bacteria, solvent action of, 141–144
bacteria, stimulation of, by salts, 137–141
calcium carbonate, effect of, on the solubility of plant-food, 127–128
carbon dioxide, effect of, on soluble plant-food of soil, 136
lime, effect of, on phosphorus, 128–129
manganese compounds, as fertilizers, 132–133
nitrogen-containing salts compared with dried blood as a source of nitrogen for plants, 122–123
phosphorus, action of salts and bacteria on, 145–151
references, 152–160
saline solutions, effect of, on phosphates, 133–136
sodium chloride as a fertilizer, 126–127
sodium nitrate and ammonium sulfate, relative value of, when used in connection with insoluble phosphate, 123–126
sulfates, as fertilizers, 129–132
summary, 151–152
bacteria oxidizing vanillin to vanillic acid, 475–485
Changes in Composition of the Soil and of the Water Extract of the Soil, Following Addition of Manure (paper), P. L. Hibbard, 259–272
alkalinity, changes in, 268–269
carbon, total, 261–262
carbon dioxide in the soil atmosphere, 261
conclusions, 271
experiments, description of, 259–260
fixation of plant-food by soil, 265–266
manure, changes in composition of, due to rotting, 268
nitrate, changes in, 267–268
nitrogen, total, at various times, 262, 264
plant-food, increase of, 269–270
references, 271–272
summary, 270
water extracts, total matter soluble in, at various times, 263, 264–265
flora, relation of, to toxicity, 60–63
Reaction, Adjusting the Soil Reaction to the Crop (editorial), J. G. Lipman, 181
solution, the concentration of, 65–69
Soils—
Note on Recent Work Concerning Acid Soils (paper), L. T. Sharp and D. R. Hoagland, 197–200
The Abnormality of, in Field-placed Cylinder Experiments (paper), C. A. Mooers, 247–251
The Influence of Heated Soils on Seed Germination and Plant Growth (paper), J. Johnson, 1–103
ammonia, production of, in heated soils, 45–59
discussion of results, 76–80
distillation, dry, of soil, 63–65
fungi, development of, in heated soils, 36–39

introduction, 1–2
literature, brief summary of, 2–5
materials and methods, 5–8
microorganisms, conditions favoring development of, in heated soils, 74–76
plant growth on heated soils, 23–36
plates, 88–103
qualitative changes in plant growth on heated soils, 33–36
soil type, 24–27
temperature of heating, 27–28
temperature of soil following sterilization, influence of, 31–32
variation with kind of plat, 28–30
references, 84–87
seed germination on heated soils, 8–22
seed type, influence of, 13–15
soil type, 10–13
temperature of heating, 10
soil flora, relation of, to toxicity, 60–63
soil solution, the concentration of, 65–69
sterilized soil, principal changes produced in and by, 3
summary, 81–84
toxic and beneficial substances produced by heating, the nature of, 39–42
toxic and beneficial properties, relation of, 69–74
water extracts of heated soils. 42–45

Solubility of potassium and other soil constituents, affected by sulfofication and nitrification, 183–195

Soybean Germination, The Effect of, upon the Growth of its Nodule-forming Bacteria (paper), A. R. C. Haas and E. B. Fred, 237–245

Soybeans, effect of inoculation and lime on yield and amount of nitrogen of, 455–467

Stable Manure, The Inhibition by, of the Injurious Effects of Alkali Salts in Soils (paper), C. B. Lipman and W. F. Gericke, 105–120.

Stewart, R., and Wyatt, F. A. (paper), The Comparative Value of Various Forms of Limestone, 273–278

Subsoils, Some Notes on the Cause of Unproductivity of "Raw" Subsoils in Humid Regions (paper), P. R. McMiller, 233–236

Sulfofication and Nitrification, Effect of, on Potassium and other Soil Constituents (paper), J. W. Ames and J. E. Boltz, 183–195
acidity of solution, 184–187
aluminum, iron, and manganese, solubility of, 194
calcium and magnesium, solubility of, 192–194
potassium solubility, 187–192
procedure, 183–184
summary, 195

Toxic and beneficial substances produced by heating soils, 39–42, 69

Truog, E., and Meacham, M. R. (paper), Soil Acidity: II. Its Relation to the Acidity of the Plant Juice, 469–474

Vanillic acid, formation of from vanillin by soil bacteria, 475–485

Vanillin, The Oxidation of, to Vanillic Acid by Certain Soil Bacteria (paper), W. J. Robbins and E. C. Lathrop, 475–485

Water extracts—
of heated soils, 42–45
total soluble matter in, 263, 264–265

Workman, A. C., Bear, F. E., and (paper), The Ammonia-fixing Capacity of Calcium Sulfate, 283–291

Wyatt, F. A., Stewart, R., and (paper), The Comparative Value of Various Forms of Limestone, 273–278

FREAS ELECTRIC OVENS

The Standard Constant Temperature Apparatus

Recognized as the most accurate, reliable, and durable. Require a minimum of current. Made in many different styles and sizes, so as to fit in with every need. Used for determining total solids in foods; moisture in wheat, flour, sugar, coal,

No. 100

ores, etc.; oxidizing qualities of linseed oil products; drying qualities of varnishes and enamels; evaporating tests of asphalt; life tests of rubber hose, etc.; experimental baking; botanical investigations; also for incubating and sterilizing. Special forms for vacuum shaking, autoclaving, conditioning, and constant humidity tests.

The Vacuum Ovens are regularly employed for the more difficult organic tests: as, for example, in connection with many colloids, flour, milk and other special foods; fertilizers; perfumes, explosives, etc.

Write for New 50-Page Catalog

giving full information about above forms, also about Freas Incubators, Tube Furnaces, Water Baths, Water Thermostats, etc.

EIMER & AMEND

FOUNDED 1851

EDUCATIONAL AND INDUSTRIAL LABORATORY SUPPLIES

Third Ave., 18th to 19th Sts. **NEW YORK CITY**

PITTSBURGH OFFICE: 2011 Jenkins Arcade

Information is the Key to Opportunity

The AGRICULTURAL INDEX reveals the opinions, experiments and progress of experts in the United States and ten foreign countries thru the indexing of articles in seventy-eight of the leading agricultural journals.

There are nine current numbers of the Index, the tenth number being a bound annual which contains in one subject alphabet all material for the year.

The new three-year cumulation, 1916–1918, contains 1,056 pages with 70,752 references.

The great future progress of the farmer, on which depends the sustenance of the peoples of the world, must be built upon knowledge of the successful results of others.
The only key to these recorded results is

THE AGRICULTURAL INDEX

1918 **Abstracts of Bacteriology** Volume II

Comprising reviews and abstracts of American and foreign work in Bacteriology, Mycology and Protozoology in their relation to the arts and sciences

Under the Editorial direction of the
SOCIETY OF AMERICAN BACTERIOLOGISTS

EDITOR
A. PARKER HITCHENS

ASSOCIATE EDITOR
GEORGE H. SMITH

Published bi-monthly. One volume a year at present. About 600 pages to a volume. Subscriptions are received for the volume only

Back volumes: Vol. I, 1917, Nos. 1–6 incl., $5.00 domestic; $5.50 foreign. Charges are net postpaid

Price: { North America, $5.00, a volume, net postpaid
Foreign, $5.50, a volume, net postpaid

Subscription Order for Abstracts of Bacteriology

WILLIAMS & WILKINS COMPANY
MOUNT ROYAL AND GUILFORD AVENUES
BALTIMORE, MD., U. S. A. ..... 191....

I / We } enclose.....for subscription to Vols. I and II ABSTRACTS OF BACTERIOLOGY

Name.....
(Please state whether Dr., Prof., etc.)

Address.....

INFORMATION FOR CONTRIBUTORS

SOIL SCIENCE is devoted to the broader outlook of the entire field of soil fertility. Articles dealing with the more important facts, observations, deductions and problems of soil biology, soil chemistry, and soil physics are published. Papers devoted to plant physiology, agronomy, bacteriology, or geology are accepted for publication if they contribute directly to the knowledge of soil fertility. The study of the mineral and organic constituents of soils, soil gases, soil water as a solvent of soil material, soil colloids, the transformation of commercial plant foods in soils, questions that deal with the fundamental facts of soil fertility and productivity, are given consideration. The phenomena concerning soil micro-organisms, such as bacteria, molds, protozoa, and algae, receive careful attention.

Manuscript submitted for publication and communications concerning editorial matters should be sent to Jacob G. Lipman, New Jersey Agricultural Experiment Station, New Brunswick, N. J., U. S. A.

Twenty-five reprints of articles will be furnished gratis to contributors when ordered in advance. A table giving cost of additional reprints, with an order slip, is sent with proof.

INFORMATION FOR SUBSCRIBERS

SOIL SCIENCE is issued monthly. Each monthly issue consists of approximately 85 pages. Two volumes a year are planned at present. Each volume consists of approximately 500 pages. Subscriptions are accepted to begin with the first number of the volume in process. The volumes for the year begin with the January and July issues, respectively. **The subscription price is $6.00 for Volumes VII** (Nos. 1-6 inclusive) **and VIII** (Nos. 1-6 inclusive) January, 1919, to December, 1919, inclusive, United States and dependencies. An additional charge of 25 cents is made for subscriptions in Canada, and 50 cents for subscriptions in foreign countries. **Back vo umes** are supplied on orders for Vols. I to VI. incl. Price, $18.00 domestic; $18.75 Canada; $19.50 elsewhere. Extra single volumes will be supplied on request when in stock at $3.00, domestic; $3.15, Canada; $3.25, elsewhere. Single numbers can be furnished in many instances at $.50 cents a copy. Prices are net, postpaid.

Correspondence concerning business matters should be addressed to Williams & Wilkins Company, Mount Royal and Guilford Avenues, Baltimore, U. S. A.

Volumes for 1919: Volumes VII and VIII.
Back volumes: Volumes I, II, III, IV, V, and VI (1916, 1917, 1918).

Subscriptions are received:

For Argentina and Uruguay: Beutelspacher y Cia, Sarmiento 815, Buenos Aires, Argentina.

For Brazil: Dr. Almeida Rego, Rua da Assembleia, 39, Rio de Janerio.

For Belgium: Henri Lamertin, 58 Rue Coudenburg, Bruxelles.

For the British Empire, except Canada: Mr. C. F. Clay, Manager, The Cambridge University Press, Fetter Lane, London, E. C. England. Subscription price, per year (Vols. VII and VIII), 28s. net, postpaid. British subscribers are requested to make checks and money orders payable to Mr. C. F. Clay, Manager, at the London address.

For Canada: Wm. Dawson & Sons, Ltd., 87 Queen Street, East, Toronto, Canada. Subscription price, per year (Vols. VII and VIII), $6.25, postpaid.

For Denmark: H. Hagerup's Boghandel, Gothersgade 30, Kobenhavn.

For Germany: R. Friedländer & Sohn, Buchhandlung, Carlstrasse 11, Berlin NW. 6.

For Holland: Scheltema & Holkema, Rokin 74–76, Amsterdam.

For Japan and Korea: Maruzen Company, Ltd. (Maruzen-Kabushiki-Kaisha), 11–15 Nihonbashi Tori-Sanchome, Tokyo; Fukuoka, Osaka, Kyoto, and Sendai, Japan. Subscription price, per year (Vols. VII and VIII), ¥17.00 net, postpaid.

For Italy: Ulrico Hoepli, Milan, Italy. Subscription price, per year (Vols. VII and VIII), $6.50 net, postpaid.

For Switzerland: Georg & Cie, Freiestrasse 10, Basel, Switzerland. Subscription price, per year (Vols. VII and VIII), $6.50 net, postpaid.

For United States and other countries except as above: Williams and Wilkins Company, Mount Royal and Guilford Avenues, Baltimore, U. S. A. Subscription price, per year (Vols. VII and VIII), United States and dependencies, $6.00 net, postpaid; Canada, $6.25 net, postpaid; foreign countries, $6.50 net, postpaid.

No claims for copies lost in the mails will be allowed unless such claims are received within 30 days (domestic), and 60 days, (foreign), of the date of issue. Claimants must state that the publication was not delivered at their recorded address. The publishers will not be responsible for loss due to change of address unless notification is received at least one week in advance of issue.

New Facts on Greenhouse Vegetable Growing

THE facts were prepared with particular consideration for the man who wants the basic money making facts about under-glass vegetable growing.

Contains growing information by Chas. Seabrook, Carl Weiant, and such leaders. Send for a copy. It's free.

Lord & Burnham Co.

Builders of Greenhouses and Conservatories

IRVINGTON New York — NEW YORK 42nd St. Bldg. — PHILADELPHIA Land Title Bldg. — CHICAGO Continental Bank Bldg.

BOSTON Little Bldg. — CLEVELAND 2063 E. 4th St. — TORONTO Royal Bank Bldg. — MONTREAL Transportation Bldg.

Eastern Factory Irvington, N. Y. — Western Factory Des Plaines, Ill. — Canadian Factory St. Catharines, Ont.

DIRECTORY OF FERTILIZER MANUFACTURERS AND DEALERS

The American Agricultural Chemical Co.

2 RECTOR STREET

Cable Address: Agrichem New York City

BAUGH & SONS CO.

Manufacturers and Importers

Office: 20 South Delaware Ave. Philadelphia, Pa.
Works: Foot of Morris St.

HAFLEIGH & COMPANY

Manufacturers of

BONE PRODUCTS

American, Somerset, Philip and Cambria Sts.
PHILADELPHIA, PA.

International Agricultural Corporation

General Offices, 61 Broadway
NEW YORK

Southern Department
Hurt Bldg. Atlanta, Ga.

LISTERS AGRI. CHEM. WORKS

NEWARK, N. J.

FERTILIZERS

Affiliated with The American A. C. Co.

N. J. Fertilizer & Chemical Co.

Works
Croxton, Jersey City, N. J.
Office
136 Water St. New York City

The Phosphate Mining Company

Manufacturers of

Acid Phosphate

124 Bay St., East Savannah, Georgia

RASIN - MONUMENTAL CO.

Subsidiary of the
VIRGINIA-CAROLINA CHEMICAL CO.
Manufacturers of
Fertilizers and Sulphuric Acid
BALTIMORE MARYLAND

Steacy & Wilton Company

Manufacturers of

"Sterling" Brand Hydrated Lime

WRIGHTSVILLE PENNA.

SWIFT & COMPANY

Producers of
BLOOD—BONE—TANKAGE
Manufacturers of
HIGH-GRADE FERTILIZERS
Harrison Station NEWARK, N. J.

Virginia-Carolina Chemical Co.

Manufacturers of
FERTILIZERS
Eastern Sales Office
Equitable Building 120 Broadway
NEW YORK CITY

CHARLES WARNER COMPANY

Manufacturers of Warner's

"LIMOID"

(*Pure "Cedar Hollow" Hydrated Lime*)
ALSO FINELY PULVERIZED LIMESTONE
Wilm'n, Del. Philada., Pa. N. Y. City

For Advertising Rates

Apply to

Williams & Wilkins Company

Publishers

Baltimore

Ice Machines

for

Dairies

BRUNSWICK REFRIGERATING COMPANY

Dept. G.

NEW BRUNSWICK, N. J.

INSURE YOUR SEED WITH

The Improved Soil Inoculator, Nobbe-Hiltner Process

NITRAGIN

TRADE MARK REG. U. S. PAT. OFF.

Restores and Maintains Soil Fertility

"NITRAGIN" is grown from original Hiltner cultures—bacteria with the greatest possible nitrogen-gathering power.

It is sold in a modern scientific form—the granular medium in the ventilated can. The bacteria are therefore of high virility at seeding time.

"NITRAGIN" is sold by seed dealers generally. If your dealer cannot supply you, order direct from

The "NITRAGIN" Co., Waterloo, Iowa

Write for free booklet

Enriches the Soil

Increases the Yield

COMMERCIAL FERTILIZER

Official Organ of the Southern Fertilizer Association

The authorized and universally circulated spokesman of the fertilizer industry in the section of the United States which consumes more than 80 per cent of all "complete" fertilizers.

A monthly journal devoted exclusively to the interests of the Fertilizer Manufacturers and Allied Industries. Articles pertaining to the scientific development of Agriculture—The production and use of fertilizers and all other means, devices, inventions and discoveries, the aim being to improve and facilitate the increase of soil production, are given principal consideration.

Subscription $1.00 per Annum
We solicit yours

WALTER W. BROWN, Publisher

623-25-26 HURT BUILDING :: ATLANTA, GA.

The

Journal of Immunology

Official Organ of the Society for Serology and Hematology and the American Association of Immunologists.

ARTHUR F. COCA, Editor

The Journal is of interest from the practical and theoretical standpoints.

Five dollars a volume—United States and Canada. Five dollars and fifty cents elsewhere.

Order from

Williams & Wilkins Co.

Baltimore, U. S. A.

Of Interest to Authors and Editors

A Plea for Coöperation

To effect greater economy in production costs and production time contributors are respectfully urged to observe the following rules when preparing manuscript for publication in this journal.

Copy. All copy should be typewritten upon white paper, 8½ x 11 inches. It should be typewritten upon one side only and sent to publishers flat, not rolled or folded.

Composition of matter in different sizes of type is done on different machines. Therefore, footnotes, reference lists, tables, quotations, and all other matter that is to be set in different type from that of the text, should be typewritten upon separate sheets.

Quotations. Quotations of four full lines or more are set in smaller type than the text proper; quotation marks are to be omitted.

Each individual quotation should be typewritten upon a separate sheet of paper.

Footnotes. While there are exceptions, as a rule, footnotes are not desirable. Descriptive or explanatory matter can almost invariably be incorporated in the text. Bibliographical references should be placed at the end of the paper under the heading of "References."

Where footnotes cannot be avoided, they should be numbered consecutively for each paper, typewritten collectively, and placed at end of manuscript. They will be placed upon proper type pages when the article is paged.

References. References are numbered consecutively in the order of their appearance in the text and are printed at the end of the article under the heading of "References." Text references to this list are indicated by numerals in parentheses instead of the superior figures used for footnotes.

The system of abbreviations adopted is that employed in the "Index-Catalogue of the Surgeon-General's Library," and the style adopted by the American Medical Association.

The author's initials are important, but not his titles or degrees. Therefore, be sure to supply the former and omit the latter.

References should be typewritten upon separate sheets of paper.

Abbreviations. Obscure and misleading abbreviations should be avoided.

As the metric system is of universal usage by research workers, it is desired that such abbreviations as "gm.," "mm.," "cc.," "mgm.," "kgm.," etc., be used in all cases when preceded by a numeral; e.g., "10 mm.," "5.05 cc." They should be spelled in full when not preceded by a numeral.

The symbol of percentage (%) is not to be used, but to be spelled as "per cent."

Tables. All tables of two or more columns are ruled. Care should be taken that as few cross rules as possible are used. Such rules add greatly to the cost of composition and in most instances a blank space will answer the same purpose.

Tables should be numbered with Arabic numerals in consecutive order from 1 up.

Each individual table should be typewritten upon a separate sheet of paper.

Illustrations. Figures should be numbered consecutively in the order in which they are to appear in the text.

Explanation and legends of figures are a part of the text and should be typewritten upon separate sheets of paper and not upon the illustrations.

Usually unmounted and untrimmed prints are preferred, but when a composition illustration is employed the prints should be pasted upon heavy cardboard and dried under pressure to prevent wrinkling.

The important feature of a photograph often occupies a few square inches in the center. If this part is indicated by light pencil marks, much expense can be saved by avoiding the reproduction of unessential backgrounds.

General. Alterations are expensive. In a few instances they are necessary, but where an author uses the proof sheets to put finishing touches upon an article, it is only reasonable that he should pay for the alterations.

From the receipt of copy by the editor to the time of actual printing, the slightest error or lack of clarity in copy is likely to cause delay and useless expense. Authors are, therefore, strongly urged to read and revise all manuscripts with the utmost care before sending same to the editor.

To aid authors in the proper preparation of manuscript we have prepared two pamphlets, "How to Prepare a Paper for Publication," and "A Brief Technical Talk." Both will be gladly sent free of charge upon request.

WILLIAMS & WILKINS COMPANY.
BALTIMORE, U. S. A.

S.S.

NOW READY FOR DISTRIBUTION

CATALOG A

APPARATUS

FOR

AGRICULTURE

AND

BIOLOGY

MANUFACTURED AND SOLD BY THE

CENTRAL SCIENTIFIC COMPANY

CHICAGO, U.S.A.

"At the head of all the sciences and arts, at the head of civilization and progress, stands—not militarism, the science that kills, not commerce, the art that accumulates wealth—but AGRICULTURE, the mother of all industry, and the maintainer of human life."—*Garfield.*

The Barrett Company

Commercial Sulphate of Ammonia | **Arcadian Sulphate of Ammonia**

Sulphate of Ammonia is the most important carrier of nitrogen in American agriculture. The possibilities of its use in mixed fertilizers and as a nitrogenous top dressing are only beginning to be realized. The production has doubled in the last five years thru new by-product ovens, yet one-half of the possible production from the coke now made is wasted every year due to the use of old beehive coke ovens. The by-product ovens will replace the beehive ovens as the demand for by-products warrant such changes.

In every state where commercial fertilizers are used, or appear likely to be needed, questions as to the best methods of using Sulphate of Ammonia in agronomy, olericulture, and horticulture, will be asked. In most cases the best answers cannot be given without experiment and investigation. Frequently tests extending over a term of years are deemed advisable. The sooner such work is put under way, the sooner helpful local data will be available. There are also many problems lying more in the region of research that require solution and invite the consideration of agricultural scientists and students.

The Barrett Company

AMMONIA SALES AGENCY DEPARTMENT
17 Battery Place, New York

AGRICULTURAL DEPARTMENT

New York, N. Y. Atlanta, Ga. Baltimore, Md.
Medina, Ohio Berkeley, Cal.

THE LIFE OF
CHILEAN NITRATE DEPOSITS

Estimated Life of Deposits at present rate of World's consumption	Upwards of 300 Years
Total Nitrate Deposits in Chile	720 Million Tons

For reliable information write

DR. WM. S. MYERS

DIRECTOR

CHILEAN NITRATE COMMITTEE

25 Madison Ave., New York